Risks of 2,4-D Use to the Federally Threatened California Red-legged Frog (*Rana aurora draytonii*) and Alameda Whipsnake (*Masticophis lateralis euryxanthus*)

Pesticide Effects Determination

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Table of Contents

List of Attachments

List of Appendices

1. Executive Summary

The purpose of this assessment is to evaluate potential direct and indirect effects on the California red-legged frog *(Rana aurora draytonii*) (CRLF) and Alameda whipsnake (*Masticophis lateralis euryxanthus*) (AW) arising from FIFRA regulatory actions regarding use of 2,4-D on agricultural and non-agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of designated critical habitat for the CRLF and AW. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) and procedures outlined in the Agency's Overview Document (U.S. EPA, 2004).

The CRLF was listed as a threatened species by USFWS in 1996. The species is endemic to California and Baja California (Mexico) and inhabits both coastal and interior mountain ranges. The AW was listed as threatened on December 5, 1997 (62 FR 64306) by the U.S. Fish and Wildlife Service (USFWS, 1997 and Westphal, 1998). The species inhabits the Inner Coast Ranges in western and central Contra Costa and Alameda counties, with occurrences additionally recorded in San Joaquin and Santa Clara counties (USFWS, 1997, 2005, and 2006).

2,4-D (2,4-Dichlorophenoxyacetic acid) is a registered herbicide used as a plant growth regulator that is available in several chemical forms (**Table 1.1**). Each of these chemical forms has multiple registered end-use products. Target pests include a wide variety of broadleaf weeds and aquatic weeds. Formulation types registered include emulsifiable concentrate, granules, soluble concentrate/solid, soluble concentrate/liquid, water dispersible granules (dry flowable), and wettable powder. Currently, labeled uses of 2,4-D include agricultural and non-agricultural uses. Among the nationally registered uses, soybean and cranberry are not grown in California, and 2,4-D is not labeled for use on strawberries in California. The uses provided in **Table 2.4** constitute the federal action evaluated in this assessment.

Table 1.1 Chemical forms of currently registered 2,4-D products

PC Code	CAS Number	Chemical Name
030001	94-75-7	2,4D acid
030004	2702-72-9	2,4D sodium salt
030016	5742-19-8	2,4D diethanolamine (DEA) salt
030019	2008-39-1	2,4D dimethylamine (DMA) salt
030025	5742-17-6	2,4D Isoproylamine (IPA) salt
030035	32341-80-3	2,4D triisopropanolamine (TIPA) salt
030053	1929-73-3	2,4D butoxyethyl ester (BEE)
030063	1928-43-4	2,4D 2 ethylhexyl ester (EHE)
030066	94-11-1	2,4D isopropyl ester (IPE)

2,4-D is an herbicide in the phenoxy or phenoxyacetic acid family that is used post-emergently for selective control of broadleaf weeds. 2,4-D, a synthetic auxin herbicide, causes disruption of plant hormone responses. Endogenous auxins are plant growth regulator hormones. These growth regulating chemicals cause disruption of multiple growth processes in susceptible plants by affecting proteins in the plasma membrane, interfering with RNA production, and changing the properties and integrity of the plasma membrane. Excessive cell division and the resulting growth destroy the plant's vascular transport system. Plant injuries include growth and reproduction abnormalities, especially on new growth, but are not limited to these. The most susceptible tissues are those that are undergoing active cell division and growth (Gibson and Liebman, 2002).

Bridging strategies to combine data across the forms of 2,4-D were established for both the environmental fate and environmental toxicity data. These strategies follow the strategies used in the 2,4-D Reregistration Eligibility Document (RED) and risk assessments conducted for other phenoxy chemicals. All fate and toxicological values have been converted to the acid equivalent (a.e.) based on the ratio of molecular weights. This was done for ease of comparing fate parameters and toxicity values across the various forms of 2,4-D. A brief summary of each strategy and rationale is given below. More detailed discussions are presented in the respective sections of this document.

EFED proposed an environmental fate strategy in the 1988 Registration Standard for bridging the degradation of 2,4-D esters and 2,4-D amine salts to 2,4-D acid. This strategy follows the strategy used in the 2,4-D RED and assessments of other related phenoxy chemicals. The bridging data provide information on the dissociation of 2,4-D amine salts and hydrolysis of 2,4-D esters. The bridging data indicate esters of 2,4-D are rapidly hydrolyzed in alkaline aquatic environments, soil/water slurries, and moist soils. The weight of evidence from open literature and registrant-sponsored data indicates that 2,4-D amine salts and 2,4-D esters are not persistent under most environmental conditions including those associated with most sustainable agricultural conditions. 2,4-D amine salt dissociation is expected to be instantaneous (< 3 minutes) under most environmental conditions. Although the available data on de-esterification of 2,4-D ester may not support instantaneous conversion from the 2,4-D ester to 2,4-D acid under all conditions, it does show 2,4-D esters in normal agriculture soil and natural water conditions are short lived compounds (half-lives < 2.9 days). To account for the potential for slower hydrolysis of the esters, acute aquatic exposure to the esters through drift+runoff, as well as runoff only, was modeled as well. Chronic exposure to 2,4-D esters was not considered since exposure is expected to be short-lived.

In concert with the fate bridging strategy, EFED established a bridging strategy for ecological toxicity of 2,4-D. Within each of these bridged groups of 2,4-D forms, the most sensitive toxicity endpoint was used for risk estimation. Toxicity data were not available for all taxa and all forms. In those cases, it was assumed that toxicity would be similar as in the other formulations in the same group.

For acute effects to aquatic animals (including aquatic-phase amphibians) and plants, data evaluating 2,4-D acid and salts have been bridged, while the data evaluating the three esters were separately bridged (**Table 1.2**). On an a.e. basis, acute toxicity to the acid and salts is comparable; however, acute toxicity to the esters tends to be two to three orders of magnitude higher. Since long-term exposure to the esters is not expected in aquatic environments, chronic risk estimation for esters, as well as the acid and salts, was conducted using chronic toxicity data based on the acid and salts.

For terrestrial animals (including terrestrial-phase amphibians) and plants, all data evaluating 2,4-D acid, salts, and esters have been bridged (**Table 1.2**). Within an organism group, the variation in the toxicity endpoints is less than two orders of magnitude, and for some groups, the variation is less than one order of magnitude.

Table 1.2 Summary of toxicity bridging strategies for 2,4-D	
Acid and Salts bridged for estimating acute toxicity to aquatic organisms and plants[a]	
PC Code	**Chemical Name**
030001	2,4D acid
030004	2,4D sodium salt
030016	2,4D diethanolamine (DEA) salt
030019	2,4D dimethylamine (DMA) salt
030025	2,4D Isoproylamine (IPA) salt
030035	2,4D triisopropanolamine (TIPA) salt
Esters bridged for estimating acute toxicity to aquatic organisms and plants	
PC Code	**Chemical Name**
030053	2,4D butoxyethyl (BEE) ester
030063	2,4D 2 ethylhexyl ester (EHE)
030066	2,4D isopropyl ester (IPE)
Acid, Salts, and Esters bridged for estimating acute and chronic toxicity to terrestrial organisms and plants	
PC Code	**Chemical Name**
030001	2,4D acid
030004	2,4D sodium salt
030016	2,4D diethanolamine (DEA) salt
030019	2,4D dimethylamine (DMA) salt
030025	2,4D Isoproylamine (IPA) salt
030035	2,4D triisopropanolamine (TIPA) salt
030053	2,4D butoxyethyl (BEE) ester
030063	2,4D 2 ethylhexyl ester (EHE)
030066	2,4D isopropyl ester (IPE)
[a]For aquatic organisms, chronic toxicity data from acid and salts also used for chronic toxicity to esters, as long-term exposure to the esters was not expected.	

The effects determinations for each listed species assessed is based on a weight-of-evidence method that relies heavily on an evaluation of risks to each taxon relevant to assess both direct and indirect effects to the listed species and the potential for modification of their designated critical habitats (*i.e.*, a taxon-level approach). Since the assessed species exist within aquatic (CRLF only) and terrestrial habitats, exposure of the listed species, their prey, and their habitats to 2,4-D are assessed separately for the two habitats[1]. Tier-II aquatic exposure models (PRZM/EXAMS) are used to estimate high-

[1] The life history of the AW (**Attachment 3**) indicates that it occupies only terrestrial habitats and consumes only terrestrial prey. For this reason, the AW was determined to be a solely terrestrial species and was not included in the aquatic portion of this assessment.

end exposures of 2,4-D in aquatic habitats resulting from runoff and spray drift from different uses. Peak model-estimated environmental concentrations resulting from different 2,4-D uses range from 0.08 to about 47 µg/L with the exception of direct aquatic applications, which result in much higher exposure estimates. These estimates are supplemented with analysis of available California surface water monitoring data from U.S. Geological Survey's National Water Quality Assessment (NAWQA) program and the California Department of Pesticide Regulation (CDPR). The maximum concentration of 2,4-D acid reported by NAWQA for California surface waters with agricultural watersheds is 1.39 µg a.e./L. This value is approximately 33 times less than the maximum model-estimated environmental concentration. The maximum concentration of 2,4-D acid reported by the CDPR surface water database (2.78 µg a.e./L) is roughly 17 times lower than the highest peak model-estimated environmental concentration.

To estimate 2,4-D exposures to terrestrial species resulting from uses involving 2,4-D applications, the T-REX model is used for foliar and granular uses. The AgDRIFT model is used to estimate deposition of 2,4-D on terrestrial and aquatic habitats from spray drift. The TerrPlant model is used to estimate exposures following foliar 2,4-D applications to terrestrial-phase CRLF and AW habitats, including plants inhabiting semi-aquatic and dry areas. The T-HERPS model is used to allow for further characterization of dietary exposures of terrestrial-phase amphibians and reptiles.

The effects determination assessment endpoints for the listed species include direct toxic effects on the survival, reproduction, and growth of the listed species itself, as well as indirect effects, such as reduction of the prey base or modification of its habitat. If appropriate data are not available, toxicity data for birds are generally used as a surrogate for reptiles and terrestrial-phase amphibians, and toxicity data from fish are used as a surrogate for aquatic-phase amphibians.

Several degradates have been identified for 2,4-D in various environmental fate studies. There is no evidence in the Reregistration Eligibility Decision (RED) document that any of these degradates are of toxicological concern, and none is found in a significant amount (>10.0%). A study in the public literature (ECOTOX) made observations of 2,4-dichlorophenol (2,4-DCP), which may be more toxic than the parent 2,4-D to earthworms; however, based on insignificant amounts (3.5% in an aerobic soil metabolism study), indirect effects to the CRLF and AW via consumption of earthworms exposed to 2,4-DCP are not of toxicological concern.

2,4-dichlorophenol (2,4-DCP) is a degradate and a key chemical intermediate in the manufacture of 2,4-D, and the purity of this intermediate has a strong correlation to the purity of 2,4-D acid produced from it. In the manufacture of 2,4-DCP, multiple positions around the phenyl ring structure may be chlorinated. The desired positions for chlorination are carbons two and four of the phenyl ring, but the reaction may yield small quantities of compounds chlorinated at different positions. Certain combinations of these chlorinated structures may form precursors to dioxin. However, according to 2,4-D registrants, since the 1990's the manufacturing process for 2,4-D and its chemical intermediate, dichlorophenol, have been modified; those modifications decrease the chance that polychloro-dibenzodioxins (PCDD) and polychloro-furans (PCDF) are formed during the manufacturing process. Based on EFED's risk assessment, dietary exposure of terrestrial organisms (birds and mammals) to chlorodibenzo-p-dioxin (CDD; dioxin) or chlorodibenzo-p-furan (CDF; furan) as contaminants in technical 2,4-D and 2,4-D ester herbicides were considered to be of no toxicological concern to piscivorous birds and mammals.

Risk quotients (RQs) are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the Agency's levels of concern (LOCs) to identify instances where 2,4-D use within the action area has the potential to adversely affect the assessed species and designated critical habitat via direct toxicity or indirect toxicity based on direct effects to its food supply or habitat. When RQs for each particular type of effect are below LOCs, the pesticide is determined to have "no effect" on the listed species being assessed. Where RQs exceed LOCs, a potential to cause adverse effects is identified, leading to a conclusion of "may affect." If a determination is made that use of 2,4-D use "may affect" the listed species being assessed and/or its designated critical habitat, additional information is considered to refine the potential for exposure and effects. Best available information is used to distinguish those actions that "may affect but not likely to adversely affect" (NLAA) from those actions that "may affect and likely to adversely affect" (LAA) for each listed species assessed. For designated critical habitat, distinctions are made for actions that are expected to have "no effect" on a designated critical habitat from those actions that have a potential to result in habitat modification.

Based on the best available information, the Agency makes a "may affect and likely to adversely affect" determination for both the CRLF and AW from the use of 2,4-D for all labeled uses except Citrus and Potatoes. For Citrus and Potatoes, the Agency makes a "may affect but not likely to adversely affect" determination for both the CRLF and AW from the use of 2,4-D.

A summary of the risk conclusions and effects determinations for the CRLF and the AW and their critical habitats are presented in **Tables 1.3** and **1.4**. Use-specific determinations for the CRLF are provided in **Table 1.5**, which also includes a summary of LOC exceedances for direct effects to the CRLF for each modeled scenario and taxonomic group. A summary of indirect effect LOC exceedances for the CRLF for each modeled scenario and taxonomic group are provided in **Table 1.6**. LOC exceedances for direct effects and indirect effects to the AW are summarized in **Tables 1.7 and 1.8.** Further information on the results of the effects determination is included as part of the Risk Description in **Section 5.2**. Given the LAA determination for the CRLF and AW and potential modification of designated critical habitat for the CRLF and AW, a description of the baseline status and cumulative effects for the CRLF is provided in **Attachment 2,** and the baseline status and cumulative effects for the AW are provided in **Attachment 4**.

Table 1.3 Effects Determination Summary for the Effects of 2,4-D on the CRLF and AW		
Assessment Endpoint	**Effects Determination** [1]	**Basis for Determination**
Survival, growth, and/or reproduction of CRLF individuals	LAA[2]	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): Freshwater fish data used as surrogate for CRLF. *Adult survival*: Acute LOC was exceeded in the aerial forestry, tree and brush control drift+runoff ester uses and all direct application to water scenarios. The chance of individual effects (*i.e*., mortality) for freshwater fish (surrogate for aquatic-phase CRLFs) is as high as ~1 in 1 for direct water applications. Out of 26 incidents reported for aquatic organisms for 2,4-D acid and DMA salt, six registered uses were reported with certainties of highly probable(2), probable(2) and possible (2). Incidents for 2,4-D were filed on aquatic organisms from runoff or drift. Use sites for the above incidents were reported on home/lawn, corn, agricultural areas, rights of way/railroad, lake, pond, stream, turf/golf course. *Growth and reproduction:* Chronic LOC was not exceeded for any scenarios.
		Terrestrial-phase (Juveniles and Adults): Avian data used as surrogate for CRLF. *Survival:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for granular applications. The chance of individual effects (*i.e*., mortality) for terrestrial-phase CRLF (Avian data used as surrogate for CRLF) is as high as ~1 in 1 for direct water applications (ditchbanks), non-cropland, forestry, tree and brush control, and grass grown for sod applications. Based on one incident report from runoff, 2,4-D has been implicated as being toxic to birds with probable certainty for a use of undetermined legality. *Growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbanks) for liquid applications derived from T-REX and T-HERPS modeled scenarios.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity *Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios. *Vascular aquatic plants*: LOC was exceeded for several acid/salt use scenarios and all direct application to water scenarios. *Freshwater invertebrates*: Acute LOC was exceeded for all direct application to water scenarios. Based on the results of probit analysis, there is a significant chance (> 10%) that direct applications to water (aquatic weed control ester uses) will impact prey of the CRLF via direct effects on aquatic invertebrates as dietary food items. *Freshwater fish*: Acute LOC was exceeded for aerial forestry, tree and brush control, and all direct application to water scenarios. Based on the results of

Table 1.3 Effects Determination Summary for the Effects of 2,4-D on the CRLF and AW		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
		probit analysis, there is a significant chance (> 10%) that direct applications to water will impact prey of the CRLF via direct effects on freshwater fish as dietary food items. Out of 26 incidents reported for aquatic organisms for 2,4-D acid and DMA salt, 7 registered uses were reported with certainties of highly probable(2), probable(2) and possible (2). Incidences for 2,4-D were filed on aquatic organisms from runoff or drift. Use sites for the above incidents were reported on home/lawn, corn, agricultural areas, rights of way/railroad, lake, pond, stream, turf/golf course.
		Terrestrial prey items, riparian habitat *Terrestrial invertebrates*: Acute LOC for small insects was exceeded for all scenarios except citrus and potatoes. Acute LOC for large insects was exceeded for several scenarios. *Terrestrial-phase amphibians, acute toxicity*: Acute LOCs were exceeded in all T-REX and T-HERPS modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for T-REX modeled granular applications. The chance of individual effects (*i.e.*, mortality) for terrestrial-phase CRLF (Avian data used as surrogate for CRLF) is as high as ~1 in 1 for direct water applications (ditchbanks), non-cropland, forestry, tree and brush control, and grass grown for sod applications. *Terrestrial-phase amphibians, growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbank exposure) for liquid application. *Small terrestrial mammals, acute toxicity:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for granular applications. Based on the results of probit analysis, there is a significant chance (> 10%) that several of the 2,4-D uses will impact prey of the CRLF via direct effects on mammals as dietary food items. Based on three incident reports, 2,4-D has been implicated as being toxic to mammals with possible and probable certainty for registered and undetermined use legalities. *Small terrestrial mammals, growth and reproduction:* For liquid applications of 2,4-D, chronic dose-based LOCs were exceeded for all application scenarios. Chronic-dietary based RQ values exceeded the LOC for all liquid application scenarios except potatoes and citrus. *Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios.

Table 1.3 Effects Determination Summary for the Effects of 2,4-D on the CRLF and AW		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
		For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. 140 of these incidents were registered uses and 143 were of unknown legality. The majority of the reports were of possible to highly probable certainty. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover.
Survival, growth, and/or reproduction of AW individuals	LAA[2]	**Potential for Direct Effects**
		Terrestrial-phase (Juveniles and Adults): Avian data used as surrogate for AW. *Survival:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for granular applications. The chance of individual effects (*i.e.*, mortality) for AW (Avian data used as surrogate for AW) is as high as ~1 in 1 for direct water application (ditchbanks). Based on one incident report 2,4-D, has been implicated as being toxic to birds with probable certainty for an undetermined use legality. *Growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbanks) for liquid application.
		Potential for Indirect Effects
		Terrestrial prey items, riparian habitat *Terrestrial invertebrates*: Acute LOC for small insects was exceeded for all scenarios except citrus and potatoes. Acute LOC for large insects was exceeded for several scenarios. *Terrestrial-phase amphibians, acute toxicity*: Acute LOCs were exceeded in all T-REX and T-HERPS modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for T-REX modeled granular applications. The chance of individual effects (*i.e.*, mortality) for terrestrial-phase CRLF (Avian data used as surrogate for CRLF) is as high as ~1 in 1 for direct water application (ditchbanks). *Terrestrial-phase amphibians, growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbank exposure) for liquid application. *Small terrestrial mammals, acute toxicity:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for granular applications. Based on the results of probit analysis, there is a significant chance (> 10%) that several of the 2,4-D uses will impact prey of the AW via direct effects on mammals as dietary food items. Based on three incident reports, 2,4-D has been implicated as being toxic to

Table 1.3 Effects Determination Summary for the Effects of 2,4-D on the CRLF and AW		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
		animals with possible and probable certainty for registered and undetermined use legalities. *Small terrestrial mammal, growth and reproduction*: For liquid applications of 2,4-D, chronic dose-based LOCs were exceeded for all application scenarios. Chronic-dietary-based RQ values exceeded the LOC for all liquid application scenarios except potatoes and citrus. *Birds, acute toxicity:* Acute LOC was exceeded in all modeled scenarios for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbank exposure) for granular applications. Based on the results of probit analysis, there is a significant chance (> 10%) that all uses except potatoes and citrus uses will impact prey of the AW via direct effects on birds as dietary food items. Based on one incident report, 2,4-D has been implicated as being toxic to animals with probable certainty for an undetermined use legality. *Birds, growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbank exposure) for liquid application. *Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. 140 of these incidents were registered uses and 143 were of unknown legality. The majority of the reports were of possible to highly probable certainty. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover.

[1] No effect (NE); May affect but not likely to adversely affect (NLAA); May affect and likely to adversely affect (LAA)

[2] The LAA call is for all usese except Citrus and Potatoes. For both Citrus and Potatoes for both species (CRLF and AW), a NLAA call was made by EFED. For Citrus and Potato, the LOC was exceeded for several indirect effects: (1) mammals as prey (chronic, CRLF and AW), (2) birds as prey (acute, AW only), and (3) terrestrial plants (CRLF and AW). The reasons for the NLAA calls are listed below:

- Although the mammalian dose-based chronic LOC was exceeded for both the CRLF and the AW prey, EFED determined that this effect would be insignificant as the potential small effect on mammal reproduction (as prey of the CRLF and AW) would not likely impact the overall prey base. It is anticipated that any effects would be small since the RQs only mildly exceeded the LOC.
- Although the avian acute dose-based LOC was exceeded for AW prey, EFED determined that this effect was discountable and insignificant as the predicted percentage of acute effect was only 0.0033% of the bird population (birds as prey items of the AW), and if even if this effect did occur, the overall prey base of the AW would likely not be affected.
- Although the terrestrial plant LOC was exceeded for both CRLF and the AW, EFED determined the effect to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.

Table 1.4 Effects Determination Summary for Critical Habitat Impact Analysis			
Species	**Assessment Endpoint**	**Effects Determination [1]**	**Basis for Determination**
CRLF	Modification of aquatic-phase PCE	HM[2]	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. 140 of these incidences are registered uses. *Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios. *Vascular aquatic plants*: LOC was exceeded for several acid/salt use scenarios and all direct application to water scenarios. There is a potential for direct effects to aquatic-phase CRLF and indirect effects via reduction of aquatic-phase prey items (aquatic invertebrates, fish, and aquatic-phase amphibians) as described in **Table 1.3** above.
	Modification of terrestrial-phase PCE	HM[2]	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. 140 of these incidences are registered uses. There is a potential for direct effects to terrestrial–phase CRLF and indirect effects via reduction of terrestrial-phased prey items (mammals, terrestrial invertebrates, and frogs) as described in **Table 1.3** above.
AW	Modification of terrestrial-phase PCE	HM[2]	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. 140 of these incidences are registered uses. There is a potential for direct and indirect effects to the AW via reduction of terrestrial-phased prey items (mammals, birds, terrestrial invertebrates, and frogs) as described in **Table 1.3** above.

[1]Habitat modification (HM) or No effect (NE)

[2] The HM call is for all usese except Citrus and Potatoes. For both Citrus and Potatoes for both species (CRLF and AW), a NE call was made by EFED. For Citrus and Potato, the LOC was exceeded for several indirect effects: (1) mammals as prey (chronic, CRLF and AW), (2) birds as prey (acute, AW only), and (3) terrestrial plants (CRLF and AW). The reasons for the NE calls are listed below:

- Although the mammalian dose-based chronic LOC was exceeded for both the CRLF and the AW prey, EFED determined that this effect would be insignificant as the potential small effect on mammal reproduction (as prey of the CRLF and AW) would not likely impact the overall prey base. It is anticipated that any effects would be small

Table 1.4 Effects Determination Summary for Critical Habitat Impact Analysis			
Species	**Assessment Endpoint**	**Effects Determination [1]**	**Basis for Determination**

since the RQs only mildly exceeded the LOC.

- Although the avian acute dose-based LOC was exceeded for AW prey, EFED determined that this effect was discountable and insignificant as the predicted percentage of acute effect was only 0.0033% of the bird population (birds as prey items of the AW), and if even if this effect did occur, the overall prey base of the AW would likely not be affected.
- Although the terrestrial plant LOC was exceeded for both CRLF and the AW, EFED determined the effect to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.

Table 1.5 2,4-D Use-specific Effects Determinations (based on direct and indirect effects) and Direct Effect LOC Exceedance Summary for the CRLF

Scenario	Method[1]	Overall Effects Determination[2]	Direct Effect LOC Exceedance			
			Aquatic Habitat		Terrestrial Habitat	
			Acute[3]	Chronic	Acute	Chronic
Orchard Uses						
Nut Orchards, Pistachios	G	LAA	No	No	Yes	No
Filbert	G	LAA	No	No	Yes	No
Grapes	G	LAA	No	No	Yes	No
Grapes (wine grapes)	G	LAA	No	No	Yes	No
Blueberries	G	LAA	No	No	Yes	No
Stone and Pome Fruits	G	LAA	No	No	Yes	No
Citrus	G	NLAA	No	No	No	No
	A	NLAA	No	No	No	No
Agricultural – Food Crop Uses						
Field Corn, Popcorn	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Sweet Corn	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Potatoes	G	NLAA	No	No	No	No
	A	NLAA	No	No	No	No
Sugarcane	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Cereal Grains	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Grain or Forage Sorghum	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Hops	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Asparagus	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Fallow Land and Crop Stubble	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Agricultural – Non-food Crop Uses						

Table 1.5 2,4-D Use-specific Effects Determinations (based on direct and indirect effects) and Direct Effect LOC Exceedance Summary for the CRLF						
Scenario	Method[1]	Overall Effects Determination[2]	Direct Effect LOC Exceedance			
			Aquatic Habitat		Terrestrial Habitat	
			Acute[3]	Chronic	Acute	Chronic
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	LAA	No	No	Yes	No
Non-agricultural Uses						
Non-cropland	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Forestry	G	LAA	No	No	Yes	No
	A	LAA	Yes*	No	Yes	No
Tree and Brush Control	G	LAA	No	No	Yes	No
	A	LAA	Yes*	No	Yes	No
Ornamental Turf	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Grass Grown for Seed and Sod	G	LAA	No	No	Yes	No
	A	LAA	No	No	Yes	No
Direct Application to Water Uses						
Rice Model	G	LAA	Yes+	No	Yes	No
	A	LAA	Yes+	No	Yes	No
Aquatic Weed Control (surface application or subsurface injection and ditchbank) 10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	G	LAA	Yes+*	No	Yes	Yes
	A	LAA	Yes+*	No	Yes	Yes
Aquatic Weed Control (surface application and ditchbank) 2 app @ 4 lb a.e./acre (21-day interval)	G	LAA	Yes+*	No	Yes	No
	A	LAA	Yes+*	No	Yes	No
Aquatic Weed Control (ditchbank application) 2 app @ 2 lb a.e./acre (30-day interval)	G	LAA	Yes*	No	Yes	No
	A	LAA	Yes*	No	Yes	No

[1] G = ground application. A = aerial application.
[2] The Effects Determination call for each individual scenario is based on results from evaluation of direct effects (this table) and indirect effects (**Table 1.6**). NE = No effect; NLAA = May affect but not likely to adversely affect; LAA = May affect and likely to adversely affect
[3] Yes+ = LOC exceeded for acid/salt runoff/drift scenario. Yes* = LOC exceeded for ester drift+runoff scenario. No LOCs exceeded for any ester drift only scenarios for direct effects.

Table 1.6 2,4-D Use-specific Indirect Effect LOC Exceedance Summary for the CRLF													
Scenario	Method[1]	Aquatic Plants[3]		Aquatic Invertebrates[3]		Terrestrial Invertebrates (Acute)	Terrestrial Plants	Aquatic-phase Frogs and Fish[3]		Terrestrial-phase Frogs[2]		Small Mammals	
		Non-vascular	Vascular	Acute	Chronic			Acute	Chronic	Acute	Chronic	Acute	Chronic[4]
Orchard Uses													
Nut Orchards, Pistachios	G	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Filbert	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Grapes	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Grapes (wine grapes)	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Blueberries	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Stone and Pome Fruits	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Citrus	G	No	No	No	No	No	Yes[5]	No	No	No	No	No	Yes[6]
	A	No	No	No	No	No	Yes[5]	No	No	No	No	No	Yes[6]
Agricultural – Food Crop Uses													
Field Corn, Popcorn	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Sweet Corn	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Potatoes	G	No	No	No	No	No	Yes[5]	No	No	No	No	No	Yes[6]
	A	No	No	No	No	No	Yes[5]	No	No	No	No	No	Yes[6]
Sugarcane	G	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Cereal Grains	G	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Grain or Forage Sorghum	G	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Hops	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes

Table 1.6 2,4-D Use-specific Indirect Effect LOC Exceedance Summary for the CRLF													
Scenario	Method[1]	Aquatic Plants[3]		Aquatic Invertebrates[3]		Terrestrial Invertebrates (Acute)	Terrestrial Plants	Aquatic-phase Frogs and Fish[3]		Terrestrial-phase Frogs[2]		Small Mammals	
		Non-vascular	Vascular	Acute	Chronic			Acute	Chronic	Acute	Chronic	Acute	Chronic[4]
Asparagus	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Fallow Land and Crop Stubble	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Agricultural – Non-food Crop Uses													
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Non-agricultural Uses													
Non-cropland	G	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Forestry	G	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	Yes*	No	Yes	No	Yes	Yes
Tree and Brush Control	G	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	Yes*	No	Yes	No	Yes	Yes
Ornamental Turf	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Grass Grown for Seed and Sod	G	No	No	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	A	No	Yes+	No	No	Yes	Yes	No	No	Yes	No	Yes	Yes
Direct Application to Water Uses													
Rice Model	G	No	Yes+	Yes+	No	Yes	Yes	Yes+	No	Yes	No	Yes	Yes
	A	No	Yes+	Yes+	No	Yes	Yes	Yes+	No	Yes	No	Yes	Yes
Aquatic Weed Control	G	Yes+*	Yes+*	Yes+*	No	Yes	NA	Yes+*	No	Yes	Yes	Yes	Yes

Table 1.6 2,4-D Use-specific Indirect Effect LOC Exceedance Summary for the CRLF													
Scenario	Method[1]	Aquatic Plants[3]		Aquatic Invertebrates[3]		Terrestrial Invertebrates (Acute)	Terrestrial Plants	Aquatic-phase Frogs and Fish[3]		Terrestrial-phase Frogs[2]		Small Mammals	
		Non-vascular	Vascular	Acute	Chronic			Acute	Chronic	Acute	Chronic	Acute	Chronic[4]
Surface application or subsurface injection for submersed weeds	A	Yes+*	Yes+*	Yes+*	No	Yes	NA	Yes+*	No	Yes	Yes	Yes	Yes
Aquatic Weed Control Surface application for floating and emergent aquatic weeds	G	Yes*	Yes+*	Yes+*	No	Yes	NA	Yes+*	No	Yes	No	Yes	Yes
	A	Yes*	Yes+*	Yes+*	No	Yes	NA	Yes+*	No	Yes	No	Yes	Yes
Aquatic Weed Control Irrigation ditchbank application	G	Yes*	Yes+*	Yes*	No	Yes	NA	Yes*	No	Yes	No	Yes	Yes
	A	Yes*	Yes+*	Yes*	No	Yes	NA	Yes*	No	Yes	No	Yes	Yes

[1]G = ground application. A = aerial application.
[2]LOC exceedances based on T-HERPS refinement for small frogs.
[3]Yes+ = LOC exceeded for acid/salt runoff/drift scenario. Yes* = LOC exceeded for ester drift+runoff scenario. No LOCs exceeded for any ester drift only scenario.
[4]LOC exceedances based on dose-based chronic risks to small mammals.
[5]Effect determined to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.
[6]Effect determined to be insignificant as the potential small effect on mammal reproduction (as prey of the CRLF) would not likely impact the overall prey base. It is anticipated that any effects would be small since the RQs only mildly exceeded the LOC.
NA – Risks of aquatic weed control uses to terrestrial plants were not estimated.

Table 1.7 2,4-D Use-specific Effects Determinations (based on direct and indirect effects) and Direct Effect LOC Exceedance Summary for the AW				
Scenario	Method[1]	Overall Effects Determination[2]	Direct Effect LOC Exceedance Terrestrial Habitat	
			Acute	Chronic
Orchard Uses				
Nut Orchards, Pistachios	G	LAA	Yes	No
Filbert	G	LAA	Yes	No
Grapes	G	LAA	Yes	No
Grapes (wine grapes)	G	LAA	Yes	No
Blueberries	G	LAA	Yes	No
Stone and Pome Fruits	G	LAA	Yes	No
Citrus	G	NLAA	No	No
	A	NLAA	No	No
Agricultural – Food Crop Uses				
Field Corn, Popcorn	G	LAA	Yes	No
	A	LAA	Yes	No
Sweet Corn	G	LAA	Yes	No
	A	LAA	Yes	No
Potatoes	G	NLAA	No	No
	A	NLAA	No	No
Sugarcane	G	LAA	Yes	No
	A	LAA	Yes	No
Cereal Grains	G	LAA	Yes	No
	A	LAA	Yes	No
Grain or Forage Sorghum	G	LAA	Yes	No
	A	LAA	Yes	No
Hops	G	LAA	Yes	No
	A	LAA	Yes	No
Asparagus	G	LAA	Yes	No
	A	LAA	Yes	No
Fallow Land and Crop Stubble	G	LAA	Yes	No
	A	LAA	Yes	No
Agricultural – Non-food Crop Uses				
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	LAA	Yes	No
Non-agricultural Uses				
Non-cropland	G	LAA	Yes	No
	A	LAA	Yes	No
Forestry	G	LAA	Yes	No
	A	LAA	Yes	No
Tree and Brush Control	G	LAA	Yes	No
	A	LAA	Yes	No
Ornamental Turf	G	LAA	Yes	No
	A	LAA	Yes	No
Grass Grown for Seed	G	LAA	Yes	No

Table 1.7 2,4-D Use-specific Effects Determinations (based on direct and indirect effects) and Direct Effect LOC Exceedance Summary for the AW				
Scenario	**Method**[1]	**Overall Effects Determination**[2]	**Direct Effect LOC Exceedance**	
			Terrestrial Habitat	
			Acute	**Chronic**
and Sod	A	LAA	Yes	No
Direct Application to Water Uses				
Rice Model	G	LAA	Yes	No
	A	LAA	Yes	No
Aquatic Weed Control Surface application or subsurface injection for submersed weeds	G	LAA	Yes	Yes
	A	LAA	Yes	Yes
Aquatic Weed Control Surface application or subsurface injection for submersed weeds	G	LAA	Yes	No
	A	LAA	Yes	No
Aquatic Weed Control Irrigation ditchbank application	G	LAA	Yes	No
	A	LAA	Yes	No

[1] G = ground application. A = aerial application.
[2] The Effects Determination call for each individual scenario is based on results from evaluation of direct effects (this table) and indirect effects (**Table 1.8**).
NE = No effect; NLAA = May affect but not likely to adversely affect; LAA = May affect and likely to adversely affect

Table 1.8 2,4-D Use-specific Indirect Effect LOC Exceedance Summary for the AW									
Scenario	Method[1]	Terrestrial Invertebrates (Acute)	Terrestrial Plants	Birds		Terrestrial-phase Frogs[2]		Small Mammals	
				Acute	Chronic	Acute	Chronic	Acute	Chronic[3]
Orchard Uses									
Nut Orchards, Pistachios	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Filbert	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Grapes	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Grapes (wine grapes)	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Blueberries	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Stone and Pome Fruits	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Citrus	G	No	Yes[4]	Yes[5]	No	No	No	No	Yes[6]
	A	No	Yes[4]	Yes[5]	No	No	No	No	Yes[6]
Agricultural – Food Crop Uses									
Field Corn, Popcorn	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Sweet Corn	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Potatoes	G	No	Yes[4]	Yes[5]	No	No	No	No	Yes[6]
	A	No	Yes[4]	Yes[5]	No	No	No	No	Yes[6]
Sugarcane	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Cereal Grains	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Grain or Forage Sorghum	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Hops	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes

Table 1.8 2,4-D Use-specific Indirect Effect LOC Exceedance Summary for the AW									
Scenario	Method[1]	Terrestrial Invertebrates (Acute)	Terrestrial Plants	Birds		Terrestrial-phase Frogs[2]		Small Mammals	
				Acute	Chronic	Acute	Chronic	Acute	Chronic[3]
Asparagus	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Fallow Land and Crop Stubble	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Agricultural – Non-food Crop Uses									
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Non-agricultural Uses									
Non-cropland	G	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Forestry	G	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Tree and Brush Control	G	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Ornamental Turf	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Grass Grown for Seed and Sod	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Direct Application to Water Uses									
Rice Model	G	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	A	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Aquatic Weed Control	G	Yes	NA	Yes	Yes	Yes	Yes	Yes	Yes

Table 1.8 2,4-D Use-specific Indirect Effect LOC Exceedance Summary for the AW									
Scenario	Method[1]	Terrestrial Invertebrates (Acute)	Terrestrial Plants	Birds Acute	Birds Chronic	Terrestrial-phase Frogs[2] Acute	Terrestrial-phase Frogs[2] Chronic	Small Mammals Acute	Small Mammals Chronic[3]
Surface application or subsurface injection for submersed weeds	A	Yes	NA	Yes	Yes	Yes	Yes	Yes	Yes
Aquatic Weed Control Surface application for floating and emergent aquatic weeds	G	Yes	NA	Yes	Yes	Yes	No	Yes	Yes
	A	Yes	NA	Yes	Yes	Yes	No	Yes	Yes
Aquatic Weed Control Irrigation ditchbank application	G	Yes	NA	Yes	No	Yes	No	Yes	Yes
	A	Yes	NA	Yes	No	Yes	No	Yes	Yes

[1]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.
[2]LOC exceedances based on T-HERPS refinement for small frogs.
[3]LOC exceedances based on dietary-based chronic risks to small mammals.
[4]Effect determined to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.
[5]Effect determined to be discountable and insignificant as the predicted percentage of acute effect was only 0.0033% of the bird population (birds as prey items of the AW), and if even if this effect did occur, the overall prey base of the AW would likely not be affected.
[6]Effect determined to be insignificant as the potential small effect on mammal reproduction (as prey of the AW) would not likely impact the overall prey base. It is anticipated that any effects would be small since the RQs only mildly exceeded the LOC.
NA – Risks of aquatic weed control uses to terrestrial plants were not estimated.

Based on the conclusions of this assessment, a formal consultation with the U.S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated.

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the listed species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (*i.e.*, attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF and AW life stages within the action area and/or applicable designated critical habitat. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the assessed species.
- Quantitative information on prey base requirements for the assessed species. While existing information provides a preliminary picture of the types of food sources utilized by the assessed species, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth, or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment and, together with the information described above, a more complete prediction of effects to individual species and potential modification to critical habitat.

2. Problem Formulation

Problem formulation provides a strategic framework for the risk assessment. By identifying the important components of the problem, it focuses the assessment on the most relevant life history stages, habitat components, chemical properties, exposure routes, and endpoints. The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (U.S. EPA, 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA, 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS, 2004).

2.1 Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the federally threatened California red-legged frog *(Rana aurora draytonii*) (CRLF) and Alameda whipsnake (*Masticophis lateralis euryxanthus*) (AW) arising from FIFRA regulatory actions regarding use of 2,4-D on a variety of agricultural and non-agricultural sites as listed in **Table 2.4**. In addition, this assessment evaluates whether use on these sites is expected to result in modification of designated critical habitat for the CRLF and AW. This ecological risk assessment has been prepared to be consistent with the settlement agreements in two court cases. This ecological risk assessment has been prepared consistent with the settlement agreement in *Center for Biological Diversity (CBD) vs. EPA et al.* (Case No. 02-1580-JSW(JL)) which addresses the CRLF and was entered in Federal District Court for the Northern District of California on October 20, 2006. This assessment also addresses the AW for which 2,4-D was alleged to be of concern in a separate suit (*Center for Biological Diversity (CBD) vs. EPA et al.* (Case No. 07-2794-JCS)).

In this assessment, direct and indirect effects to the CRLF and AW and potential modification to designated critical habitat for the CRLF and AW are evaluated in accordance with the methods described in the Agency's Overview Document (U.S. EPA, 2004). The effects determinations for each listed species assessed is based on a weight-of-evidence method that relies heavily on an evaluation of risks to each relevant taxon to assess both direct and indirect effects to the listed species and the potential for modification of their designated critical habitats (*i.e.*, a taxon-level approach). Screening level methods include use of standard models such as PRZM-EXAMS, T-REX, TerrPlant and AgDRIFT, all of which are described at length in the Overview Document. Additional refinements include an analysis of the usage data, a spatial analysis, and use of the T-HERPS model. Use of such information is consistent with the methodology described in the Overview Document (U.S. EPA, 2004), which specifies that "the assessment process may, on a case-by-case basis, incorporate additional methods, models, and lines of evidence that EPA finds technically appropriate for risk management objectives" (Section V, page 31 of U.S. EPA, 2004).

In accordance with the Overview Document, provisions of the ESA, and the Services' *Endangered Species Consultation Handbook,* the assessment of effects associated with registrations of 2,4-D is based on an action area. The action area is the area directly or indirectly affected by the federal action, as indicated when the Agency's Levels of Concern (LOCs) are exceeded. It is acknowledged that the action area for a national-level FIFRA regulatory decision associated with a use of 2,4-D may potentially involve numerous areas throughout the United States and its territories. However, for the purposes of this assessment, attention will be focused on relevant sections of the action area including those geographic areas associated with locations of the CRLF and AW and their designated critical habitats within the state of California. As part of the "effects determination," one of the following three conclusions will be reached for each of the assessed species in the lawsuits regarding the potential use of 2,4-D in accordance with current labels:

- "No effect";
- "May affect but not likely to adversely affect"; or
- "May affect and likely to adversely affect".

The CRLF and AW have designated critical habitats associated with them. Designated critical habitat identifies specific areas that have the physical and biological features, known as primary constituent elements (PCEs), essential to the conservation of the listed species. The PCEs for CRLFs are aquatic and upland areas where suitable breeding and non-breeding aquatic habitat is located, interspersed with upland foraging and dispersal habitat. The PCEs for the AW are scrub/shrub communities with a mosaic of open and closed canopy, woodland or annual grassland plant communities, and lands containing rock outcrops, talus, and small mammal burrows.

If the results of initial screening-level assessment methods show no direct or indirect effects (no LOCs are exceeded) to individuals or to the PCEs of the species' designated critical habitat, a "no effect" determination is made for use of 2,4-D as it relates to each species and its designated critical habitat. If, however, potential direct or indirect effects to individuals of each species are anticipated or effects may impact the PCEs of the designated critical habitat, a preliminary "may affect" determination is made for the FIFRA regulatory action regarding 2,4-D.

If a determination is made that use of 2,4-D "may affect" a listed species or its designated critical habitat, additional information is considered to refine the potential for exposure and for effects to each species and other taxonomic groups upon which these species depend (*e.g.*, prey items). Additional information, including spatial analysis (to determine the geographical proximity of the assessed species' habitat and 2,4-D use sites) and further evaluation of the potential impact of 2,4-D on the PCEs is also used to determine whether modification of designated critical habitat may occur. Based on the refined information, the Agency uses the best available information to distinguish those actions that "may affect but are not likely to adversely affect" from those actions that "may affect and are likely to adversely affect" the assessed listed species, including potential modification to the PCEs of its designated critical habitat. This information is presented as part of the Risk Characterization in **Section 5** of this document.

The Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because 2,4-D is expected to directly impact living organisms within the action area (defined in **Section 2.7**), critical habitat analysis for 2,4-D is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes (*i.e.*, the biological resource requirements for the listed species associated with the critical habitat or important physical aspects of the habitat that may be reasonably influenced through biological processes). Activities that may modify critical habitat are those that alter the PCEs and appreciably diminish the value of the habitat. Evaluation of actions related to use of 2,4-D that may alter the PCEs of the assessed species' critical habitat form the basis of the critical habitat impact analysis. Actions that may affect the assessed species' designated critical habitat have been identified by the Services and are discussed further in **Section 2.6**.

2.2 Scope

2,4-D is a plant growth regulator most commonly used as a herbicide for control of broadleaf weeds. It is produced in multiple chemical forms (see **Table 1.1** and **Figure 2.1**). After review of all the available data, EFED developed bridging strategies for both the fate and toxicity components of the assessments. These strategies are detailed in **Section 2.4.1** and **Section 2.8.1**. 2,4-D is an ingredient in many agricultural and home use products. It exists in these products as either the sole active ingredient or as an active ingredient working in conjunction with other active ingredients. Target pests include a wide variety of broadleaf weeds and aquatic weeds. Registered formulation types include emulsifiable concentrate, granules, soluble concentrate/solid, water dispersible granules (dry flowable), and wettable powder.

The end result of the EPA pesticide registration process (*i.e.*, the FIFRA regulatory action) is an approved product label. The label is a legal document that stipulates how and where a given pesticide may be used. Product labels (also known as end-use labels) describe the formulation type (*e.g.*, liquid or granular), acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. Thus, the use or potential use of 2,4-D in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Although current registrations of 2,4-D allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of 2,4-D in portions of the action area that are reasonably assumed to be biologically relevant to the CRLF and AW and their designated critical habitats. Further discussion of the action area for the CRLF and AW and their critical habitat is provided in **Section 2.7**.

Bridging strategies to combine data across the forms of 2,4-D were established for both the environmental fate and environmental toxicity data. These strategies follow the strategies used in the 2,4-D RED and risk assessments conducted for other phenoxy chemicals. All fate and toxicological values have been converted to the acid equivalent (a.e.) based on the ratio of molecular weights. This was done for ease of comparing fate parameters and toxicity values across the various forms of 2,4-D. A brief summary of each strategy and the reasoning is given below. More detailed discussions are presented in the respective sections of this document.

EFED utilized an environmental fate strategy in the 1988 Registration Standard for bridging the degradation of 2,4-D esters and 2,4-D amine salts to 2,4-D acid. The bridging data provides information on the dissociation of 2,4-D amine salts and hydrolysis of 2,4-D esters. To account for the potential for slower hydrolysis of the esters, acute aquatic exposure to the esters through runoff and drift, as well as runoff only, was modeled in addition to acute and chronic exposure to the acid and salts. Chronic exposure to 2,4-D esters was not considered since exposure is expected to be short-lived.

In concert with the fate bridging strategy, EFED established a bridging strategy for ecological toxicity of 2,4-D. Within each of these bridged groups of 2,4-D forms, the most sensitive toxicity endpoint was used for risk estimation. For acute effects to aquatic animals (including aquatic-phase amphibians) and plants, data evaluating 2,4-D acid and salts have been bridged, while the data evaluating the three esters was separately bridged. Since long-term exposure to the esters is not expected in aquatic environments, chronic risk estimation for esters, as well as the acid and salts, was conducted using chronic toxicity data based on the acid and salts. For terrestrial animals (including terrestrial-phase amphibians) and plants, all data evaluating 2,4-D acid, salts, and esters have been bridged. Within an organism group, the variation in the toxicity endpoints is less than two orders of magnitude, and for some groups, the variation is less than one order of magnitude.

Several degradates were identified for 2,4-D in various environmental fate studies. There is no evidence in the Reregistration Eligibility Decision (RED) document that any of these degradates are of toxicological concern, and none of them is found in a significant amount (>10.0%). The Metabolism Assessment Review Committee (MARC) determined that all residues other than the parent 2,4-D are not of risk concern due to low occurrence under environmental conditions, comparatively low toxicity, or a combination thereof (W. Hazel and L. Taylor, TXR No. 0052264, D293119, 12/3/03). Two studies evaluating the degradate, 2,4-dichlorophenol (2,4-DCP), were found in open literature. 2,4-DCP was found to be more toxic to earthworms than the parent 2,4-D acid with LC_{50}'s of 61.6 (95% CI: 41.0-92.4) $\mu g/cm^2$ and 4.4 (95% CI: 3.2-5.9) $\mu g/cm^2$ in a 48-hr study (Roberts and Dorough 1984, E040531). In a second study conducted on male Swiss mice, results indicated that the genotoxic effect of 2,4-DCP was weaker than that of 2,4-D based on chromosomal aberrations and sperm-head abnormalities (E93505; Amer and Aly, 2001). As with previous assessments conducted by the Agency, this assessment will be based on the parent 2,4-D only.

The Agency's risk assessments do not routinely contain an evaluation of mixtures of active ingredients including either mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the Agency's Overview Document and the Services' Evaluation Memorandum (U.S. EPA, 2004; USFWS/NMFS, 2004).

2,4-D has registered products that contain multiple active ingredients. Analysis of the available open literature and acute oral mammalian LD_{50} data for multiple active ingredient products relative to the single active ingredient is provided in **Appendix A.** Based on a review of the available studies on 2,4-D mixtures in ECOTOX, the information presented does not indicate that 2,4-D mixtures are more toxic than the single active ingredient. Therefore, the results of this analysis show that an assessment based on the toxicity of the single active ingredient of 2,4-D is appropriate.

2.3 Previous Assessments

The Environmental Protection Agency issued the final Registration Eligibility Decision (RED) for 2,4-D in June 2005. EFED's chapter, "Revised Environmental Fate and Effects Division Revised Preliminary Risk Assessment for the 2,4-Dichlorophenoxyacetic acid (2,4-D) Reregistration Eligibility Decision Document," was finalized on October 29, 2004. EFED concluded use of 2,4-D on terrestrial sites presents the greatest potential risks to non-target terrestrial plants, mammals, and birds, while the use of 2,4-D for aquatic weed control presents risk to aquatic organisms and plants. According to the Required Labeling Changes section in the final Agency RED (signed June 30, 2005), many of the changes were related to user safety requirements, such as PPE (personal protective equipment), REI (restricted entry interval). For use-specific application restrictions, a setback of greater than or equal to 600 ft may be required for the protection of drinking water. However, those requirements will not change the outcomes of this effects determination. In addition, label rate changes for some uses and spray drift management requirements were established as part of the Required Labeling Changes in the RED. The spray drift management requirements were designed to limit the conditions on droplet size, wind speed, temperature inversions, and equipment, and they are expected to be effective in reducing the off-target spray drift.

The Agency also reviewed the registrant's endangered species assessment for 2,4-D (Review of registrant submission entitled "Endangered Species Assessment on Non-Target Plants Potentially at Risk from Use of 2,4-Dichlorophenoxyactic (*sic*) Acid in Almonds, Rice, Strawberries, and Wheat," August 26, 2005). EFED concluded that this assessment does not include sufficient documentation to support the findings of "no effect" for most of the listed plant species initially identified as "potential concern" by the co-occurrence process and the county-level resolution analysis.

An endangered species assessment to determine the potential risks to 26 listed ESUs of Pacific salmon and steelhead was done by the Field and External Affairs Division (FEAD) in 2004 ("2,4-Dichlorophenoxyacetic Acid Analysis of Risks to Endangered and Threatened Salmon and Steelhead," December 1, 2004). This assessment was based on the draft 2004 EFED RED chapter. FEAD determined that the acid and salts are practically non-toxic to slightly toxic to fish, the esters are slightly toxic to highly toxic to fish and moderately toxic to freshwater invertebrates, and all forms are highly toxic to vascular plants. Terrestrial uses in the Pacific Northwest pose no direct or indirect risks to fish. However, acid and salt uses may cause indirect risks to fish via applications to rice crops or for aquatic weed control; esters may cause acute and chronic risks directly and indirectly via aquatic weed control uses. As a result, FEAD determined that terrestrial crop usage would have no effect on the 26 listed ESUs of Pacific salmon and steelhead. Rice uses may affect but are not likely to adversely affect (NLAA) 4 ESUs and would have no effect on 22 ESUs. For aquatic weed control uses, usage information for analysis of each ESU was deficient, but 2,4-D use may affect all 26 ESUs.

An additional endangered species assessment done by FEAD determined the potential risks of 2,4-D EHE to one listed Pacific salmonid ESU ("2,4-D ethylhexyl ester Analysis of Risks to Endangered and Threatened Salmon and Steelhead," May 7, 2004). It was determined that there would be no effect on Pacific anadromous Coho salmon due to the rapid degradation of 2,4-D EHE to the acid form.

2.4 Stressor Source and Distribution

2.4.1. Environmental Fate Bridging Strategy

The environmental fate strategy for 2,4-D is based on bridging the degradation of 2,4-D esters and 2,4-D amine salts to 2,4-D acid (Registration Standard for 2,4-Dichlorophenoxyacetic acid (2,4-D), 1988, 540/RS-88-115) (**Figure 2.1**). The bridging data provides information on the time of dissociation of 2,4-D amine salts and rate of hydrolysis of 2,4-D esters. There are acceptable bridging data for 2,4-D DMA, 2,4-D IPA, 2,4-D TIPA, 2,4-D EHE, 2,4-D BEE, 2,4-D DEA, 2,4-D IPE. The sodium salt of 2,4-D is considered to be equivalent to 2,4-D acid. The bridging data indicate esters of 2,4-D are rapidly hydrolyzed in alkaline aquatic environments, soil/water slurries, and moist soils and that the 2,4-D amine salts have been shown to dissociate rapidly in water. Under extremely dry soil conditions, these degradation mechanisms may be inhibited to increase persistence of 2,4-D esters. The laboratory bridging data indicate that under most environmental conditions, 2,4-D esters and 2,4-D amine salts will degrade rapidly to form 2,4-D acid.

Figure 2.1 Schematic of Bridging Strategy of 2,4-D amine salts and 2,4-D esters to 2,4-D acid

<u>Amine Salts of 2,4-D</u>
2,4-D-DMA
2,4-D-IPA
2,4-D-TIPA
2,4,D-DEA

dissociation in water

<u>Amines</u>
Dimethylammonium
Ispropylammonium
Triisopropylammonium
Diethanolammonium

<u>2,4-D Acid</u>

<u>Esters of 2,4-D</u>
2,4-D EHE
2,4-D BEE
2,4-D IPE

microbial-mediated hydrolysis
alkaline-catalyzed hydrolysis
surface-catalyzed hydrolysis

<u>Alcohols</u>
Ethylhexanol
Butoxyethanol
Isopropanol

Additional data submitted subsequent to establishment of the environmental fate bridging strategy generally support the strategy for the amine salts. Direct evidence of the stability of 2,4-D amine salts in soil and aquatic environments is difficult due to the lack of analytical methods. Based on maximum application rates for 2,4-D amine salts (4 lb a.e./A), 2,4-D amine salts are expected to fully dissociate in soil environments because their theoretical concentrations in soil solution does not exceed water solubilities. Additionally, dissociation studies indicate the time for complete dissociation is rapid (< 3 minutes). Although the analytical methods in the field studies for 2,4-D DMA were not capable of separating and identifying 2,4-D DMA from 2,4-D acid, the most conservative half-lives of 2,4-D DMA would be equivalent to the 2,4-D acid half-lives in field studies. Half-lives of 2,4-D (either acid or DMA) in 2,4-DMA field studies ranged from 1.1 days to 30.5 days with a median half-life of 5.6 days.

The de-esterification of 2,4-D esters is more difficult to generalize because it is dependent on heterogeneous hydrolysis (microbial-mediated and surface-catalyzed hydrolysis) and homogenous hydrolysis (alkaline catalyzed) (Schwarzenbach *et al.*,1993). The de-esterification of 2,4-D ester leads to formation of 2,4-D acid and an associated alcohol moiety. Unlike the physical dissociation mechanism of 2,4-D amine salts, the de-esterification of 2,4-D esters is dependent on abiotic and microbial-mediated processes. Any environmental variable influencing microbial populations or microbial activity could theoretically influence the persistence of the 2,4-D ester. Soil properties including clay mineralogy, organic carbon content, temperature, and moisture content are known to influence hydrolysis rates (Wolfe *et al.,* 1989 and Wolfe, 1990).

Paris *et al.* (1981) found the average de-esterification half-life of 2,4-D BEE in natural waters from 31 sites with varying temperature and pH conditions (5.4 to 8.2) was 2.6 hours. They found that 2,4-D BEE degradation could be explained using second-order kinetics accounting for microbial population numbers and aqueous concentration of 2,4-D BEE. Further research indicated second-order de-esterification rates can be predicted through a linear regression [$\log k_b=(0.799\pm0.098)* \log K_{ow} - (11.643\pm0.204)$ $r^2=0.94$] using the octanol:water coefficient ($\log K_{ow}$) as the independent variable.

Additionally, various mineral surfaces (Fe, Al, Ti oxides) have been shown to influence hydrolysis of carboxylate esters (Torrent and Stone, 1994). Abiotic hydrolysis of 2,4-D esters, however, is expected to be more predictable in alkaline environments. Several field studies show phenoxy herbicide esters are more persistent under extremely dry soil [< soil wilting point (~15 bars)] conditions (Smith and Hayden, 1980; Smith, 1972; Smith, 1976). In moist soils [~50 to 80% field capacity (~0.3 bars)] and soil slurries, phenoxy herbicide esters degraded rapidly (>85% degradation) during a 48-hour incubation period. These hydrolysis studies indicate the alkyl chain configuration affected hydrolysis rates in soils and soil slurries. The isooctyl ester of 2,4-D (2,4-D EHE) had slower hydrolysis rates when compared to n-butyl and isopropyl esters of 2,4-D. In field studies, Harrison *et al.* (1993) found no detections of 2,4-D and 2,4-DP esters in runoff water (although detection limits were relatively high @ 20 µg a.e./L for 2,4-D EHE) from turf sites where 2,4-DP and 2,4-D esters were applied.

Registrant-sponsored research indicates the 2,4-D esters (ethylhexyl, isopropyl, butoxyethyl) degrade rapidly ($t_{1/2}$< 24 hours) in soil slurries, aerobic aquatic environments, and anaerobic, acidic aquatic environments. In terrestrial field dissipation studies, the half-lives for 2,4-D EHE ranged from 1 to 14 days with median half-life of 2.9 days. 2,4-D BEE, applied as granules, degraded rapidly in the water column in aquatic field dissipation studies under alkaline conditions. However, the 2,4-D BEE residues were detected in sediment samples from Day 0 (immediately post-treatment) to 186 days post-treatment. It is unclear whether 2,4-D BEE persistence in sediment is due to the slow release of the granule formulation or to slow de-esterification of sediment-bound 2,4-D BEE. Available open literature and registrant-sponsored laboratory data would suggest slow granule dissolution prolonged the persistence of 2,4-D BEE. In forest dissipation studies, the 2,4-D EHE ester degraded slowly on foliage and in leaf litter.

The weight of evidence from open literature and registrant-sponsored data indicates that 2,4-D amine salts and 2,4-D esters are not persistent under most environmental conditions including those associated with most sustainable agricultural conditions. 2,4-D amine salt dissociation is expected to be instantaneous (< 3 minutes) under most environmental conditions. Although the available data on de-esterification of 2,4-D ester may not support instantaneous conversion from the 2,4-D ester to 2,4-D acid under all conditions, it does show 2,4-D esters in normal agriculture soil and natural water conditions are short-lived compounds (< 2.9 days). Under these conditions, the environmental exposure from 2,4-D esters and 2,4-D amine salts is expected to be minimal in both terrestrial and aquatic environments. Further analysis is required due to 2,4-D BEE persistence in sediments from aquatic field studies. Additionally, the persistence of 2,4-D EHE on foliage and in leaf litter from registrant-submitted forest field dissipation studies requires additional investigation. No field dissipation data (terrestrial, forest, or aquatic) have been submitted for the amine salts, 2,4-D IPA, 2,4-D

TIPA, and 2,4-D DEA, or for the esters 2,4-D BEE (aquatic field dissipation data is available for this chemical form) and 2,4-D IPE to confirm their persistence under field conditions.

2.4.2 Physical and Chemical Properties of 2,4-D Acid

The physical and chemical properties of 2,4-D acid are provided in **Table 2.1,** and the ratio of molecular weights for all salts and esters are provided in **Table 2.2**. Chemical structures are illustrated in **Figure 2.2**. Based on these physical and chemical properties alone, 2,4-D acid has low potential to volatilize from soils (vapor pressure) or from water (Henry's Law Constant). It is also unlikely to bioaccumulate in fish given the low value of the Log *n*-octanol/water partition coefficient. **Appendix B** provides the structures and further chemical/molecular information on 2,4-D. The molecular structure characteristics of 2,4-D acid are important as they help understanding its mode of action at a molecular level as well as the binding of 2,4-D acid to soil/sediment particulates.

Table 2.1 Physical and Chemical Properties of 2,4-D acid	
Common name	2,4-D acid
Chemical name	2,4-Dichlorophenoxyacetic acid
Molecular formula	$C_8H_6Cl_2O_3$
CAS Number	94-75-7
Molecular weight	221.04
Physical state	white crystalline solid
Melting point	138 - 141 ^oC
Vapor pressure	1.47×10^{-7} mm Hg @25 ^0C
Henry's Law	4.74×10^{-10} atm-m3/mol @ 25^0C
Solubility	569 mg/L @ 20^oC
Log K_{ow}	2.81

Table 2.2 Molecular Weight Ratios (relative to 2,4-D acid)		
PC code	**Chemical name**	**Molecular Weight Ratio**
030001	2,4D acid	1.00
030004	2,4D sodium salt	1.10
030016	2,4D diethanolamine (DEA) salt	1.48
030019	2,4D dimethylamine (DMA) salt	1.20
030025	2,4D isopropylamine (IPA) salt	1.27
030035	2,4D triisopropanolamine (TIPA) salt	1.87
030053	2,4D butoxyethyl ester (BEE)	1.45
030063	2,4D 2 ethylhexyl ester (EHE)	1.51
030066	2,4D isopropyl ester (IPE)	1.19

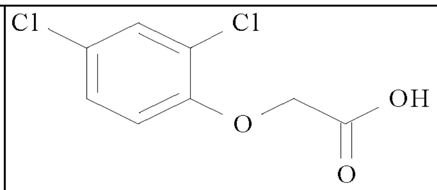
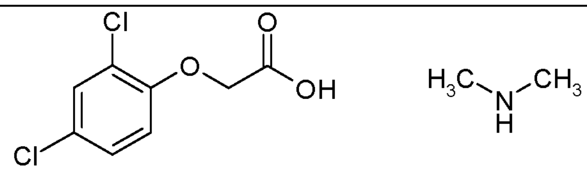
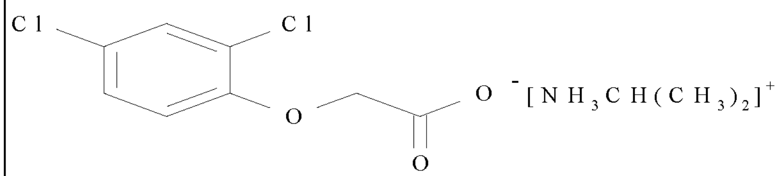
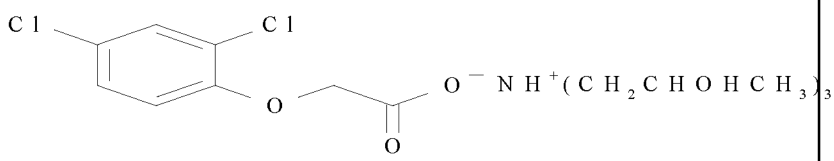
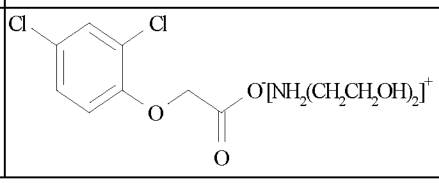
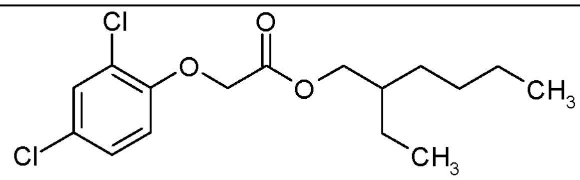
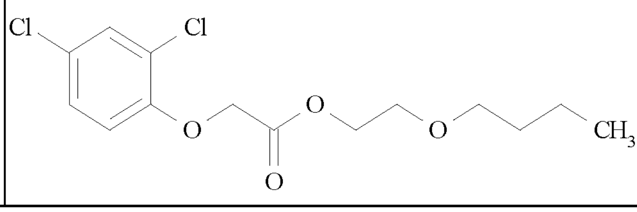
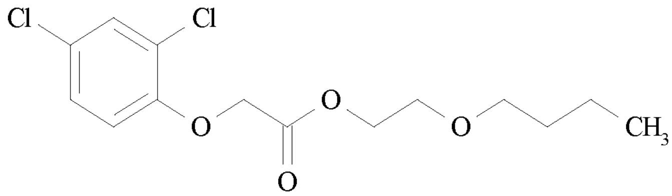
Figure 2.2 Chemical structures of all evaluated 2,4-D forms	
Acid and Sodium Salt:	
PC 0300001 (2,4-D) 2,4-dichlorophenoxyacetic acid 030004 (Na) Sodium Salt of 2,4-D	 *for Sodium Salt, "Na" replaces "H".*
Amine Salts:	
PC 030019 (DMA) Dimethylamine Salt of 2,4-D	
PC 030025 (IPA) Isopropylamine Salt of 2,4-D	
PC 030035 (TIPA) Triisopropanolamine Salt of 2,4-D	
PC 030016 (DEA) Diethanolamine Salt of 2,4-D	
Esters:	
PC 030063 (2-EHE) 2-Ethylhexyl Ester of 2,4-D	
PC 030053 (BEE) Butoxyethyl Ester of 2,4-D	

Figure 2.2 Chemical structures of all evaluated 2,4-D forms	
PC 030066 (IPE) Isopropyl Ester of 2,4-D	

2.4.3 Environmental Fate Properties of 2,4-D Acid

A complete database has been assembled for 2,4-D acid. **Table 2.3** lists the environmental fate properties of 2,4-D acid, along with the major and minor degradates detected in the submitted environmental fate and transport studies. The dissipation of 2,4-D acid appears to be dependent on oxidative microbial-mediated mineralization, photodegradation in water, and leaching. 2,4-D acid is stable to abiotic hydrolysis. Photodegradation of 2,4-D acid was observed ($t_{1/2}$=12.9 calendar days or 7.57 days of constant light) in pH 5 buffer solution. However, the 2,4-D acid photodegradation half-life on soil was 68 calendar days. Photodegradates of 2,4-D were identified as 1,2,4-benezenetriol (37% of applied) and CO_2 (25% of applied).

2,4-D acid is non-persistent ($t_{1/2}$=6.2 days) in terrestrial environments. Soil degradates were 2,4-DCP and 2,4-dichloroanisol (2,4-DCA). The half-life of 2,4-D acid in aerobic aquatic environments was 15 days. Degradates in sediment/water test systems were 2,4-dichlorophenol, 4-chlorophenol, 4-chlorophenoxyacetic acid, and chlorohydroquinone. The major volatile degradate in soil and aquatic environments was CO_2. Unidentified radio-labeled residues were detected in non-labile soil organic matter fractions (*e.g.*, fulvic acid, humic acid, and humin). Unaltered 2,4-D acid was detected in fulvic acid fractions of the soil organic matter.

2,4-D acid was moderately persistent to persistent ($t_{1/2}$=41 to 333 days) in anaerobic aquatic laboratory studies. Intermediate degradates were 2,4-DCP, 4-chlorophenol , and 2-chlorophenol. Volatile degradates were identified as CO_2, 2,4-DCA, and 4-chlorophenol.

As noted above, several degradates were detected in the laboratory fate studies reviewed. The degradates detected were 1,2,4-benzenetriol, 2,4-DCP, 2,4-DCA, chlorohydroquinone (CHQ), 4-chlorophenol, volatile organics, bound residues, and carbon dioxide. 1,2,4-benzenetriol is a photodegradate that was observed under abiotic conditions and is less likely to occur under natural conditions where microbially-mediated degradation occurs.

2,4-D acid has a low binding affinity (Kads < 3 and Kdes < 1) in mineral soils and sediment. The mobility of 2,4-D acid in supplemental soil thin layer chromatography (TLC) studies was classified as intermediately mobile (Rf=0.41) to very mobile

(Rf=1.00) in "sieved" mineral soils. Aged radio-labeled residues of 2,4-D appeared to be immobile in supplemental soil column studies.

2,4-D acid was studied in sandy loam, sand, silty clay loam, and loam soil. Freundlich Kads values were 0.17 for the sandy loam soil, 0.36 for the sand soil, 0.52 for the silty clay loam soil, and 0.28 for the loam soil. Corresponding K_{oc} values were 70, 76, 59 and 117 mL/g. 2,4-DCP had Freundlich Kads values were 2.0 for the sandy loam soil, 1.7 for the sand soil, 3.3 for the silty clay loam soil, and 2.9 for the loam soil. Corresponding K_{oc} values were 821, 368, 374 and 1204 mL/g. 2,4-DCA had Freundlich Kads values of 1.6 for the sandy loam soil, 2.1 for the sand soil, 5.4 for the silty clay loam soil, and 3.5 for the loam soil. Corresponding K_{oc} values were 667, 436, 616 and 1442 mL/g.

Table 2.3 Summary of 2,4-D Acid Environmental Fate Properties

Study	Value	Major Degradates *Minor Degradates*	MRID	Study Status
Hydrolysis	Stable		410073-01	Acceptable
Direct Aqueous Photolysis	$t_{1/2}$ = 12.98 days	1,2,4-benzenetriol (37% of applied) and CO_2 (25% of applied)	411253-06	Acceptable
Soil Photolysis	$t_{1/2}$ = 68 days	CO_2 (5% of applied)	411253-05	Acceptable
Aerobic Soil Metabolism	$t_{1/2}$ ranged from 1.44 to 12.4 days	2,4-DCP (3.5%) and 2,4-DCA (2.8%)	00116625 431675-01	Acceptable
Anaerobic Aquatic Metabolism	$t_{1/2}$ = 41 to 333 days	2,4-DCP, 4-chlorophenol, and 2-chlorophenol	433560-01, 415579-01	Acceptable
Aerobic Aquatic Metabolism	$t_{1/2}$ = 15 days	2,4-DCP, 4-chlorophenol, 4-chlorophenoxyacetic acid, and chlorohydroquinone	420453-01, 429792-01, 441886-01	Acceptable
Adsorption/ Desorption $K_{d\text{-}ads}$ / $K_{d\text{-}des}$ (mL/g) $K_{oc\text{-}ads}$ / $K_{oc\text{-}des}$ (mL/g)	Freundlich Kd values were 0.17 for the sandy loam soil, 0.36 for the sand soil, 0.52 for the silty clay loam soil, and 0.28 for the loam soil. Corresponding Koc values were 70, 76, 59, and 117 mL/g.		420453-02, 00112937, 441179-01	Acceptable
Terrestrial Field Dissipation	The first-order half-lives ranged from 1.1 days to 42.5 days with a median half-life of 6.1 days		439147-01, 437624-01, 437624-02, 435146-01, 435334-01, 438640-01, 435928-01, 437624-03, 437624-04, 436406-01, 438317-02, 438727-03, 438491-02, 438317-01, 437052-02	Acceptable
Aquatic Field	Estimated dissipation half		439083-02, 439547-01,	Acceptable

Table 2.3 Summary of 2,4-D Acid Environmental Fate Properties				
Study	**Value**	**Major Degradates** *Minor Degradates*	**MRID**	**Study Status**
Dissipation	lives of 20.7 and 2.7 days in water from the North Carolina pond after the first and second applications, 14 days and 6.1 days in water from a North Dakota pond after the first and second applications, and 1.0 day in water from the Louisiana rice paddy after the single application		434916-01	

2.4.4 Terrestrial Field Dissipation Study Summaries for 2,4-D

In order to address the field behavior of 2,4-D under actual use conditions, 15 terrestrial field dissipation studies were conducted using 2,4-D DMA, and 15 terrestrial field dissipation studies were conducted using 2,4-D EHE. No terrestrial field dissipation studies were conducted using 2,4-D IPA, 2,4-D TIPA, 2,4-D DEA, 2,4-D BEE, or 2,4-D IPE. Field studies were conducted using 2,4-D DMA on bareground, pasture, corn, turf, and wheat. Field studies were conducted using 2,4-D EHE on bareground, pasture, corn, turf, and wheat to represent major uses of 2,4-D. In addition, three aquatic field dissipation studies and one forest field dissipation study were conducted using 2,4-D DMA, while two forest field dissipation studies were conducted using 2,4-D EHE. An additional aquatic dissipation study was conducted using 2,4-D BEE.

The registrant conducted a total of 30 terrestrial field dissipation studies in CA, CO, NC, ND, NE, OH, and TX on bareground plots as well as plots cropped to corn, pasture, turf, and wheat. The first-order 2,4-D acid half-lives ranged from 1.1 days to 42.5 days with a median half-life of 6.1 days. These half-lives reflect dissipation from the surface soil layer (0 to 6 inches) and do not include residues that have leached below the surface layer. The data indicate a rapid to moderately rapid dissipation rate for 2,4-D acid. Dissipation rates for 2,4-D degradation products (2,4-DCP and 2,4-DCA) were not estimated because of their sporadic occurrence patterns in surface soils. The results of this study are also consistent with half-lives from laboratory studies. Results from laboratory studies indicate rapid to moderately rapid degradation under aerobic soil conditions with half-lives ranging from 1.4 days to 12.4 days with a median half-life of 2.9 days.

EFED believes that little information on the behavior of 2,4-D DMA and 2,4-D EHE will be gained from the submission of additional field dissipation studies. Sufficient data has been presented that demonstrates 2,4-D has a moderate to high potential for soil mobility under normal agricultural practices. 2,4-D residues were detected below a depth of 18 inches in eleven of the terrestrial field dissipation studies reviewed and was detected below 30 inches in five studies (MRID 43914701, 43762402, 43831703, 43849101, and

43872702). Leaching appears to be a route of dissipation when precipitation or irrigation exceeds evapotranspiration demands. NAWQA data reported maximum 2,4-D concentrations in surface and groundwater of 15 and 14.8 µg a.e./L, respectively. It should be noted that the next highest concentration detected in the NAWQA groundwater data is 4.54 µg a.e./L while the highest concentration detected in drinking water derived from groundwater reported in the US EPA Office of Water′s NCOD is 8 µg a.e./L.

EFED conducted comparative analysis of all 2,4-D acid half-lives estimated from the 30 field dissipation studies reviewed. Comparisons were done between granular formulations versus concentrates, between bare soil and cropped fields, and between the 2,4-D acid half lives from studies conducted with 2,4-D DMA and 2,4-D EHE forms separately. Each analysis is discussed below and all half-lives are for 2,4-D acid:

- Comparison of descriptive statistics for the granular versus concentrate half-lives suggests that the granular applications will result in longer half-lives than the concentrate forms. The granular half-lives ranged from a maximum of 24.6 days to a minimum of 5.1 days with a median half-life of 11.9 days, while the concentrate form had half-lives ranging from a maximum of 42.5 days to a minimum of 1.1 days with a median half-life of 5.5 days. The median granular half-life is approximately twice the concentrate form suggesting a longer half-life.

- Comparison of descriptive statistics for the bare soil half-lives versus cropped plot half-lives suggests that there is no appreciable difference in dissipation rates based on the presence of plants (including turf). The bare soil half-lives ranged from a maximum of 42.5 days to a minimum of 1.1 days with a median half-life of 5.1 days, while the cropped half-lives ranged from a maximum of 39.2 days to a minimum of 2.2 days with a median half-life of 7.8 days.

- Comparison of descriptive statistics for the 2,4-D acid half lives determined when applying 2,4-D DMA versus the applying 2,4-D EHE chemical form suggests that there is no appreciable difference in dissipation rates between 2,4-D DMA and 2,4-D EHE forms. The 2,4-D acid half-lives from the 2,4-D DMA studies ranged from a maximum of 30.5 days to a minimum of 1.1 days with a median half-life of 5.6 days, while the 2,4-D acid half lives from studies using the 2,4-D EHE form had half-lives ranging from a maximum of 42.5 days to a minimum of 1.2 days with a median half-life of 6.2 days.

2.4.5 Aquatic Field Dissipation Study Summaries for 2,4-D

In order to address the behavior of 2,4-D in aquatic water systems a series of aquatic field dissipation studies were conducted. Three studies were conducted using 2,4-D DMA while a fourth study was conducted using 2,4-D BEE. Two additional dispersion and dissipation studies using 2,4-D DMA were also submitted.

In three supplemental aquatic field dissipation studies conducted in North Dakota, North Carolina, and Louisiana, 2,4-D DMA immediately converted to 2,4-D acid. EFED estimated a 2,4-D half-life in water from the North Carolina pond after the first application of 20.4 days and after the second application of 2.7 days. EFED estimated a half-life of 2,4-D in water from the North Dakota pond after the first application of 14.0 days and after the second application of 6.1 days. EFED estimated a half life in water from the Louisiana rice paddy after the single application of 1.0 day. The aquatic dissipation studies for 2,4-D DMA confirm that 2,4-D DMA quickly converts to 2,4-D acid and dissipates rapidly from the water column.

In addition, the 2,4-D Task Force submitted two dispersion and dissipation studies for the application of 2,4-D DMA to control aquatic weeds. The first study was for the surface application of 2,4-D DMA to a lake in Lake Woodruff, Florida for the control of water hyacinth. The review of the study is currently pending. However, a preliminary summary of the results is presented below along with the previously reviewed studies. In this study, 2,4-D DMA was surface applied at a rate of 3.8 lb a.e./acre to approximately 3.9 acres within an overall water body of 2200 acres. The highest single concentration detected was 270 μg a.e./L at three hours after application within the application area. The highest concentration detected outside the application area was 122 μg a.e./L approximately 18.4 meters from the application area. The study authors calculated a dissipation half-life for 2,4-D from the application area of 2.3 days, however, this half-life does not distinguish between degradation, sorption, and transport away from the application area.

In the second dispersion and dissipation study, 2,4-D DMA was applied by subsurface injection to a water body located in Green Lake, Minnesota for the control of Eurasian water milfoil. 2,4-D was applied as 2,4-D DMA by subsurface injection at a rate of 10.8 pounds of acid equivalent per acre-foot (lb a.e./acre-foot) to achieve a target concentration in the application area of 4 parts per million (ppm). 2,4-D DMA was applied on September 11, 2002 to approximately 4.5 acres with a dense stand of Eurasian water milfoil. Green Lake is located in Chisago County, Minnesota. It is a 1714 acre "low-flow" lake. The study authors report that the location, test site (static to low-flow lake) and application method were chosen because they represent a typical use pattern for 2,4-D DMA. The highest single concentration detected was 13,193 μg a.e./L at one hour after application within the application area. The highest concentration detected outside the application area was 3374 μg a.e./L approximately immediately outside the application area. The furthest detection of 2,4-D outside the application area greater than the MCL was on day 11 at 82.3 μg a.e./L while the furthest concentration detected above the LOQ was 1605 meters. The study authors calculated a dissipation half life for 2,4-D from the application area of 3.23 days; however, this half-life does not distinguish between degradation, sorption, and transport away from the application area.

In a supplemental study, the aquatic field dissipation of 2,4-D BEE was studied in ponds in North Carolina, Minnesota, and Washington. A single aquatic field dissipation study conducted on three separate ponds was submitted for 2,4-D BEE. All three ponds used in this study were alkaline (pH ranged from 7.9 to 8.1). As noted in the environmental fate assessment, the esters of 2,4-D convert to 2,4-D acid by abiotic hydrolysis; however, the rate is pH dependent. 2,4-D BEE was detected in water and sediment in these studies;

however, 2,4-D BEE was not present for a sufficient time to estimate half-lives in water. Half-lives for 2,4-D acid in water from the three ponds ranged from 2 to 40 days, while the half-lives of 2,4-D acid in sediment ranged from 5 to 29 days. EFED also estimated half-lives in sediment from the North Carolina pond of 9.6 days for 2,4-D BEE and 80.5 days for the degradate 2,4-DCP. Data from this aquatic field dissipation study in granular form in the North Carolina pond suggest that the granular formulation of BEE is more persistent than the DMA chemical form. The maximum concentration detected of 2,4-D acid in water was 2,700 µg a.e./L at 15 days post-treatment from the North Carolina site.

Additional data on the behavior of 2,4-D BEE in aquatic systems was submitted in a supplemental anaerobic aquatic metabolism study. Radio-labeled 2,4-D BEE, at 7 µg/g, had a first-order half-life of 14.4 hours in a strongly acidic, rice paddy water and sediment test system. The major degradate of 2,4-D BEE was 2,4-D. The degradate 2,4-D was stable during a 12 month incubation period. Unidentified residues were also detected (<4% of applied) in sediment and water samples. The reported results suggest that 2,4-D BEE should not persist in acidic, anaerobic aquatic environments.

Finally, four aquatic field dissipation studies were previously submitted and reviewed, which provide additional information on the behavior of 2,4-D in field environments. These studies were submitted and reviewed previously as part of the Registration Standard issued in 1988. These studies provided supplemental data on the aquatic field dissipation and accumulation in non-target organisms of 2,4-D DMA and 2,4-D BEE. 2,4-D acid, formulated as Weedar 64 and applied at 20 and 40 lb/A, had a field dissipation half-life of < 3 days in reservoirs at Banks Lake, Washington and Fort Cobb, Oklahoma. In the Rock Ranch canal and the Cherry Creek lateral, 2,4-D had half-life of < 133 minutes for locations 7 miles downstream from the application site. In the Guntersville reservoir on the Tennessee River amended with 2,4-D DMA at 20 and 40 lb/A, the water concentration of 2,4-D was 4.8 µg/mL at 8 hours post-treatment and declined to <0.11 µg/mL at 6 months post-treatment. In two ponds, a bayou, a lagoon, and a lake (located in Louisiana) amended with 2,4-D DMA at 1, 4, or 10 lb/A, 2,4-D "residues" had a dissipation half-life of < 14 days. The concentration of 2,4-D residues at 7 days post-treatment ranged from 8 to 999 µg/L and then declined to 1 to 45 µg/L at 28 days post-treatment.

2.4.6 Forest Field Dissipation Study Summaries for 2,4-D

In order to address the behavior of 2,4-D in forest systems, two forest field dissipation studies were conducted. One study was conducted using 2,4-D DMA, while the second was conducted using 2,4-D EHE. In a supplemental forest field dissipation study in Oregon, 2,4-D DMA also converted rapidly to 2,4-D acid. Parent 2,4-D DMA broadcast applied as a spray (by helicopter) at a nominal rate of 4.0 lb a.e./A onto a forest plot of loam soil planted with fir trees dissipated with EFED-estimated half-lives for 2,4-D acid using linear regression of log transformed data (mean concentrations of data from 0 to 6 inches collected through 398 days) of 59 days ($r^2 = 0.74$) in exposed soil, 68 days ($r^2 = 0.63$) in protected soil, 42 days ($r^2 = 0.81$) on foliage, and 72 days ($r^2 = 0.82$) on leaf litter. In a supplemental forest field dissipation study in Georgia, parent 2,4-D EHE was broadcast applied as a spray at a nominal rate of 4.0 lb a.e./A to a forested plot of sandy clay loam soil in Georgia. EFED attempted to estimate half-lives of 2,4-D and 2,4-D EHE in soil (exposed and protected) using linear regression of log transformed data (mean concentrations of data from 0 to 6 inches collected through 398 days); however, the half-lives of 2,4-D acid in soil are questionable due to variability in the data. EFED estimated half-lives in foliage for 2,4-D of 32.5 days (r^2 = 0.80) and for 2,4-D EHE of 32.7 days (r^2 = 0.51). EFED estimated half-lives in leaf litter for 2,4-D of 51.7 days (r^2 = 0.55) and for 2,4-D EHE of 50.5 days (r^2 = 0.53).

A series of fate studies were submitted for the moieties of various chemical forms of 2,4-D. These moieties included dimethylamine (DMA), isopropylamine (IPA), triisopropylamine (TIPA), diethanolamine (DEA), ethylhexyl ester (EHE), butoxyethanol (BEE), and isopropanol (IPE). Fate studies were conducted for aerobic soil metabolism, aerobic aquatic metabolism, and anaerobic aquatic metabolism. The studies indicated that under aerobic soil conditions DMA degraded with half-lives between 4 and 14 days, EHE degraded with a half-life of 5.3 hours, IPA degraded with half-lives between 11.8 to 18.2 hours, TIPA degraded with half-lives between 0.9 to 1.6 days, BEE degraded with half-lives between 13.3 to 35.5 hours, DEA degraded with a half-life of 1.7 days, and IPE degraded with half-life of 0.9 hours. The studies indicated that under aerobic aquatic conditions, DMA degraded with a half-life of 2.8 days, IPA degraded with a half-life of 21.6 hours, TIPA degraded with a half-life of 14.3 days, BEE degraded with half-lives between 0.6 to 3.4 days, DEA degraded with a half-life of 5.8 days, and IPE degraded with a half-life of 13 hours. Finally, the studies indicated that under anaerobic aquatic conditions DMA degraded with a half-life of 1732 days, EHE degraded with a half-life of 15.3 days, IPA degraded with a half-life of 408 days, TIPA degraded with a half-life of 15.3 days, BEE degraded with a half-life of 1.4 days, DEA degraded with a half-life of 10.9 days, and IPE degraded with a half-life of 14.55 days. These data suggest that degradation products of 2,4-D moieties should not accumulate under normal agricultural conditions.

2.4.7 Environmental Transport Mechanisms

Potential transport mechanisms include pesticide surface water runoff, spray drift, and secondary drift of volatilized or soil-bound residues leading to deposition onto nearby or more distant ecosystems. Surface water runoff and spray drift are expected to be the major routes of exposure for 2,4-D.

In general, deposition of drifting or volatilized pesticides is expected to be greatest close to the site of application. Computer models of spray drift (AgDRIFT and/or AGDISP) are used to determine potential exposures to aquatic and terrestrial organisms via spray drift.

The processes by which pesticides may be transported away from the target site include spray drift at the time of application and volatilization. Spray drift has been well studied and the Agency spray drift exposure assessment is considered in EFED's risk assessment models. However, transport after volatilization is not as well studied and the impact of the potential transport of 2,4-D esters away from the target site is not included quantitatively in this assessment.

Much evidence reported in open literature suggests concern for impact to non-target organisms due to drift and volatilization of the ester forms of the phenoxy herbicides. The state of Florida passed the Organo-Auxin Herbicide Rule which restricts the use of highly volatile esters based on concerns over volatility; however, these banned esters are high volatility esters and do not include 2,4-D EHE and BEE (email from Dale Dubberly, Florida Department of Agriculture and Consumer Services, dated August 12, 2003). Other states have similarly banned or restricted the use of certain phenoxy herbicides including esters, while other states have issued warnings on the use of phenoxy herbicides, particularly under dry moisture conditions and warmer temperatures (Feitshans, 1999). A March 2008 memo from the Pesticide Registration and Evaluation Committee of DPR titled "Prioritization and status of active ingredients for risk characterization: Report 50" (http://www.cdpr.ca.gov/docs/risk/priot.pdf), lists 2,4D salt and ester compounds as Toxic Air Contaminants (TACs), and places them in high priority for review and completion of a risk assessment. Finally, the Association of American Pesticide Control Officials (AAPCO) report in the 1999 Pesticide Enforcement Survey (http://aapco.ceris.perdue.edu/doc/surveys/drift99.html) that 2,4-D is the most commonly confirmed active ingredient by state agencies as regards to drift complaints. However the survey does not distinguish between 2,4-D chemical forms, does not differentiate between drift and volatility, and indicates that the most common confirmation technique is visual examination and residue confirmation.

Data collected in the 1960s and 1970s, and summarized in Majewski and Capel (1995), indicate that 2,4-D has been detected in rainwater samples at concentrations between 50 nanograms per liter (ng/L) and 204,000 ng/L, while 2,4-D was detected in air samples at concentrations between 1.15 nanograms per gram (ng/g) and 1410 ng/g. Majewski and Capel noted that the higher concentrations were infrequently detected, and the authors also noted that the high detections were located near areas where pesticides were applied and may have resulted from unusual conditions. More recent data reported by Anderson *et al.* (2002) on water and rainfall samples in a wetland environment in Alberta, Canada indicate that 2,4-D was one of the most frequently detected pesticides in rainfall samples with a frequency of detection of 65%. However, concentrations did not exceed 1 μg/L. In a study conducted in southern Manitoba by Rawn *et al.* (1999), 2,4-D was detected in rainfall at concentrations less than 1 μg/L and was detected in air as both vapor and particle phase at a maximum concentration of 3500 picograms per cubic meter (pg/m^3). Both rainfall and air detections were closely associated with local use; however, the authors noted that the relative contribution of these compartments to surface water was low compared to runoff.

An important consideration resulting from these data is that any analysis of surface water monitoring data cannot distinguish between sources of contamination. In other words, the analysis of surface water concentrations discussed below cannot distinguish the source of the contaminant whether it be from runoff, drift, or deposition from rainfall. The reported value likely includes all sources of input into the surface water body, and thus, the effect of volatilization of 2,4-D in the aquatic exposure scenarios is lessened.

However, the impact of volatilization and the potential impact on off-site, non-target terrestrial organisms in unknown and cannot be quantified.

To assess the potential for 2,4-D to partition into various media, EFED performed an estimation of partitioning of 2,4-D acid and 2,4-D EHE with a simple fugacity model in USEPA EpiSuite software. The fugacity model predicts that the relative percentage of 2,4-D acid that will partition into air is 0.37 percent while the relative percentage for 2,4-D EHE is 0.48 percent. The results of the fugacity model suggest that for 2,4-D acid and 2,4-D EHE that volatilization is not predicted to be a major route of exposure. Uncertainties associated with the use of a fugacity model are that partitioning of 2,4-D esters to soil is estimated and that the effect of intercept and volatilization from plant surfaces is not accounted for. These facts could result in an underestimation of the amount partitioning to air.

It is noted that EFED's current risk assessment does account for spray drift as a process effecting exposure through the use of PRZM/EXAMS and the drift component. However, longer-range transport coupled with volatility and ultimately deposition via rainfall is not accounted for in this assessment and lends additional uncertainty to the risk assessment.

2.4.8 Mechanism of Action

2,4-D is a plant growth regulator in the phenoxy or phenoxyacetic acid family. It is most commonly used as a post-emergence herbicide for selective control of broadleaf weeds. 2,4-D, a synthetic auxin herbicide, causes disruption of plant hormone responses. Endogenous auxins are plant growth regulator hormones. These growth-regulating chemicals cause disruption of multiple growth processes in susceptible plants by affecting proteins in the plasma membrane, interfering with RNA production, and changing the properties and integrity of the plasma membrane. Excessive cell division and the resulting growth destroy the plant's vascular transport system. The most susceptible tissues are those that are undergoing active cell division and growth (Gibson and Liebman, 2002).

Plant injuries include growth and reproduction abnormalities, especially on new growth. Broadleaf plants experience stem and petiole twisting (epinasty), leaf malformations (parallel venation, leaf strapping, and cupping), undifferentiated cell masses and adventitious root formation on stems, and stunted root growth. Rolled leaves (onion leafing), fused brace roots, leaning stems, and stalk brittleness are effects observed on grass plants. Disruption of reproductive processes may occur resulting in sterile or multiple florets and nonviable seed production. Symptoms may appear on young growth almost immediately after application, but death may not occur for several weeks.

2.4.9 Use Characterization

Analysis of labeled use information is the critical first step in evaluating the federal action. The current labels for 2,4-D represent the FIFRA regulatory action; therefore, labeled use and application rates specified on the labels form the basis of this assessment. The June 6, 2005 version of the Master Label, prepared by SRRD and supported by the 2,4-D Industry and IR-4 (**Appendix N**), was used for use characterization and modeling scenario selection. The Master Label provided by SRRD does include mitigation regarding rates and uses that resulted from the RED. EFED utilized the rates and uses as provided in the Master Label as a detailed analysis of the current label rates was unavailable. It is possible that not all 2,4-D labels have been updated to match the rates and specifics on the Master Label. For those instances in which rates on the Master Label are lower than rates on currently used labels, EFED's risk assessment may underestimate the exposure and risk to the assessed species. In addition, SRRD has stated that the Master Label is limited by the lack of inclusion of the following:

- EPA precautionary label statements
- Worker Protection Standard information other typical REIs
- Complete recommendations and limitations related to efficacy, plant varieties, *etc.*
- Ranges of rates, mixing directions, weed lists, *etc.* as per actual labels
- Comprehensive equipment details
- A very few Section 24(c) use parameters.

The assessment of use information is critical to the development of the action area and selection of appropriate modeling scenarios and inputs.

Target pests include a wide variety of broadleaf weeds and aquatic weeds. Formulation types registered include emulsifiable concentrate, granules, soluble concentrate/solid, soluble concentrate/liquid, water dispersible granules (dry flowable), and wettable powder. 2,4-D may be applied with a wide range of application equipment including aircraft, backpack sprayer, band sprayer, boom sprayer, granule applicator, ground, hand-held sprayer, helicopter, injection equipment, tractor-mounted granule applicator, and tractor-mounted sprayers. Methods of application of 2,4-D may include band treatment, basal spray treatment, broadcast, frill treatment, girdle treatment, ground spray, soil band treatment, soil broadcast treatment, spot treatment, stump treatment, tree injection treatment, and water-related surface treatment. 2,4-D application can be applied at emergence, before bud break, at a dormant stage, at a dough stage, to established plantings, foliarly, at post-emergence, at pre-emergence, at pre-harvest, and/or at pre-plant.

The current labeled uses for 2,4-D as shown in **Table 2.4** constitute the federal action evaluated in this assessment: Soybean and cranberry are on the Master Label; however, they are not grown in California, so they are not included in this assessment. Strawberries are included on the Master Label, but are off-labeled for 2,4-D use in California, so they are not included in this assessment.

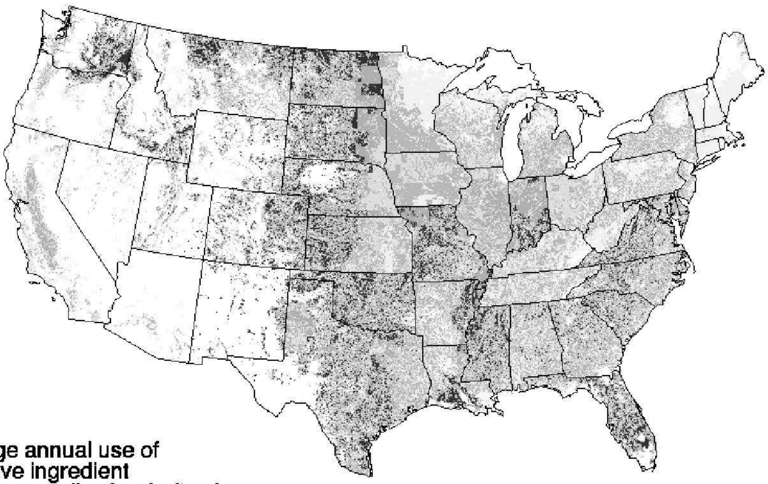
Table 2.4 2,4-D Uses Assessed for California as derived from Master Label for Reregistration of 2,4-Dichlorophenoxyacetic Acid Uses developed by SRRD			
Master Label Use Category and Detailed Uses	**Label Uses**	**Method[1]**	**Application Rate (interval between applications)**
Orchard Uses			
Nut Orchards, Pistachios	Acid, DMA, TIPA, IPA, DEA, Na	G	2 apps @ 2 lb a.e./acre (30-day interval)
Filberts	Acid, DMA, TIPA, IPA, DEA, Na	G	4 apps @ 0.5 lb a.e./acre[2] (30-day interval)
Grapes	Acid, DMA, TIPA, IPA, DEA, Na	G	1 app @ 1.36 lb a.e./acre
Grapes (wine grapes)	Acid, DMA, TIPA, IPA, DEA, Na	G	1 app @ 1.36 lb a.e./acre
Blueberries	Acid, DMA, TIPA, IPA, DEA, Na	G	1 post-emergence app @ 1.4 lb a.e./acre and 1 post-harvest app @ 1.4 lb a.e./acre
Stone and Pome Fruits	Acid, DMA, TIPA, IPA, DEA, Na	G	2 apps @ 2 lb a.e./acre (75-day interval)
Citrus	IPE	G	1 app @ 0.1 lb a.e./acre
		A	1 app @ 0.1 lb a.e./acre
Agricultural – Food Crop Uses			
Field Corn, Popcorn	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)
		A	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)
Sweet Corn	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	1 app @ 1 lb a.e./acre March 15; 1 app @ 0.5 lb a.e./acre April 29
		A	1 app @ 1 lb a.e./acre on March 15; and 1 app @ 0.5 lb a.e./acre on April 29
Potatoes Fresh market only	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	2 apps @ 0.07 lb a.e./acre (10-day interval)
		A	2 apps @ 0.07 lb a.e./acre (10-day interval)
Sugarcane[4]	Acid, DMA, TIPA, IPA, DEA, Na	G	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre (20-day interval)
		A	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre (20-day interval)
Cereal Grains Wheat, Barley, Millet, Oats, Rye	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)
		A	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)
Grain or Forage Sorghum	Acid, DMA, TIPA, IPA, DEA, Na	G	1 post-emergence app @ 1.0 lb a.e./acre
		A	1 post-emergence app @ 1.0 lb a.e./acre
	2-EHE, BEE	G	1 post-emergence app @ 0.5 lb a.e./acre

Table 2.4 2,4-D Uses Assessed for California as derived from Master Label for Reregistration of 2,4-Dichlorophenoxyacetic Acid Uses developed by SRRD			
Master Label Use Category and Detailed Uses	**Label Uses**	**Method[1]**	**Application Rate (interval between applications)**
		A	1 post-emergence app @ 0.5 lb a.e./acre
Hops	Acid, DMA, TIPA, IPA, DEA, Na	G	3 apps @ 0.5 lb a.e./acre (30-day interval)
		A	3 apps @ 0.5 lb a.e./acre (30-day interval)
Asparagus	Acid, DMA, TIPA, IPA, DEA, Na	G	2 apps @ 2 lb a.e./acre (30-day interval)
		A	2 apps @ 2 lb a.e./acre (30-day interval)
Fallowland and Crop Stubble	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	2 apps @ 2 lb a.e./acre (30-day interval)
		A	2 apps @ 2 lb a.e./acre (30-day interval)
Agricultural – Non-food Crop Uses			
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	2 apps @ 2 lb a.e./acre (30-day interval)
Non-agricultural Uses			
Non-cropland Fencerows, Hedgerows, Roadsides, Ditches, Rights-of-way, Utility power lines, Railroads, Airports, Industrial sites, and Other non-crop areas	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	1 app @ 4 lb a.e./acre
		A	1 app @ 4 lb a.e./acre
Forestry Forest site preparation, Forest roadsides, Brush control, Established conifer release including Christmas trees and reforestation areas	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	1 app @ 4 lb a.e./acre
		A	1 app @ 4 lb a.e./acre
Tree and Brush Control Alder, Ash, Aspen, Birch, Blackgum, Cherry, Elm, Oak, Sweetgum, Tulip poplar, Willow, and Others	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	1 app @ 4 lb a.e./acre
		A	1 app @ 4 lb a.e./acre
Ornamental Turf Golf courses, Cemeteries, Parks, Sports fields, Turfgrass, Lawns and other grass areas	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	2 apps @ 1.5 lb a.e./acre (21-day interval)
		A	2 apps @ 1.5 lb a.e./acre (21-day interval)
Grass Grown for Seed and Sod	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	G	2 apps @ 2 lb a.e./acre (21-day interval)
		A	2 apps @ 2 lb a.e./acre (21-day interval)
Direct Application to Water Uses			
Rice	Acid, DMA, TIPA,	G & A	1 app @ 1.5 lb a.e./acre

Table 2.4 2,4-D Uses Assessed for California as derived from Master Label for Reregistration of 2,4-Dichlorophenoxyacetic Acid Uses developed by SRRD			
Master Label Use Category and Detailed Uses	**Label Uses**	**Method[1]**	**Application Rate (interval between applications)**
	IPA, DEA, Na		
Aquatic Weed Control Surface application or subsurface injection for submersed weeds	Acid, DMA, TIPA, IPA, DEA, Na, BEE	G & A	1 app @ 10.8 lb a.e./acre foot
Aquatic Weed Control Irrigation ditchbank application	Acid, DMA, TIPA, IPA, DEA, Na, BEE	G & A	2 app @ 2.0 lb a.e./acre (30-day interval)
Aquatic Weed Control Surface application for floating and emergent aquatic weeds	Acid, DMA, TIPA, IPA, DEA, Na, BEE	G & A	2 app @ 4.0 lb a.e./acre (21-day interval)

[1] G = ground application. A = aerial application.
[2] The Master Label indicates a maximum single application rate of 1.0 lb a.e./100 gallons spray for filberts, SRRD verified that this rate is equivalent to a maximum single application rate should of 0.5 lb a.e./acre, which represents a conservative estimate.

A national map **(Figure 2.3)** showing the estimated poundage of 2,4-D agricultural uses across the United States is provided below. The map was downloaded from a U.S. Geological Survey (USGS), National Water Quality Assessment Program (NAWQA) website (http://water.usgs.gov/nawqa/pnsp/usage/maps/). All registered uses and applications are not necessarily included in this figure (*e.g.*, direct water application).



Crops	Total pounds applied	Percent national use
pastureland	21947880	55.18
wheat for grain	5443483	13.69
other hay	2820947	7.09
corn	2496503	6.28
cropland in summer fallow	1550082	3.90
soybeans	1504323	3.78
sugarcane	1426739	3.59
barley for grain	561492	1.41
sorghum	554666	1.39
rice	525714	1.32

Figure 2.3 2,4-D Use in Total Pounds per County. *This map does not necessarily include all registered uses, e.g., direct water application.*

According to 2002 annual agricultural use estimates, the total 2,4-D use was about 39.8 million pounds. Among them, pastureland use was the dominant one (55.18%). The other major uses were as follows: wheat for grain (13.69%), other hay (7.09%), corn (6.28%), cropland in summer fallow (3.90%), soybean (3.78%), sugarcane (3.59%), barley for grain (1.41%), sorghum (1.39%) and rice (1.32%).

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (Kaul and Jones, 2006) using state-level usage data obtained from USDA-NASS[2], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database[3].

[2] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.

[3] The California Department of Pesticide Regulation's Pesticide Use Reporting database provides a census of pesticide applications in the state. See http://www.cdpr.ca.gov/docs/pur/purmain.htm.

CDPR PUR is considered a more comprehensive source of usage data than USDA-NASS or EPA proprietary databases, and thus, the usage data reported for 2,4-D by county in this California-specific assessment were generated using CDPR PUR data. Eight years (1999-2006) of usage data were included in this analysis. Data from CDPR PUR were obtained for every reported pesticide application made on every use site at the section level (approximately one square mile) of the public land survey system. BEAD summarized these data to the county-level by site, pesticide, and unit area treated. Calculating county-level usage involved summarizing across all applications made within a section and then across all sections within a county for each use site and for each pesticide. The county-level usage data that were calculated include average annual pounds applied, average annual area treated, and average and maximum application rate across all eight years.

From 1999 to 2006, 2,4-D was used on 97 crops or sites in 58 counties in California. For all technical forms (*e.g.*, acid, DMA, TIPA), PUR data were reported in lb a.i./acre. Usage values reported in this assessment were also reported as lb a.i./acre. The PUR database reported usage data individually for each of the technical forms; no usage data were recorded for 2,4-D sodium salt and 2,4-D IPA. Reported usage data is a summation of usage data for each form on the basis of lb a.i./acre; no conversion to lb a.e./acre was performed. Usage data is summarized for each crop category on the Master Label (**Table 2.5**) and for each county (**Table 2.6**). Data for each individual PUR use category is provided in **Appendix C**.

The herbicide was used in the greatest quantity on wheat with an average yearly application of ~106,200 lb a.i./year. Almond, right-of-ways, uncultivated, landscape maintenance and oat followed with ~70,400 lb a.i./year, ~37,700 lb a.i./year, ~24,500 lb a.i./year, ~22,700 lb a.i./year, and ~21,100 lb a.i./year, respectively. The highest average application rate for any single use over the eight year period was 5.71 lb a.i./acre applied to regular pest control. The greatest quantity of 2,4-D was applied in San Joaquin County with a yearly average application of ~40,400 lb a.i./year followed by Merced, Kings, Imperial and Solano counties with ~33,700 lb a.i./year, ~33,100 lb a.i./year, ~33,0700 lb a.i./year, and ~28,300 lb a.i./year, respectively. The highest average county application rate over the eight year period was 2.83 lb a.i./acre applied in Alpine County; however, only 2.83 lb was applied in total in this county.

Almost of all the highest single application rates recorded in the 1999 to 2006 CDPR PUR data greatly exceed the maximum application rates permitted on 2,4-D labels and likely indicate data entry errors in the pounds applied or the acres treated data fields. The 95^{th} and 99^{th} percentile estimations of application rates aggregated by cropping category were, for the majority, less than the maximum labeled rates.

Typically, the average application rate (based on many records from the data set) is far below the maximum label-permitted application rate. For instance, the average application rate for wheat (0.73 lb a.i./acre) was only 58.4 % of the maximum labeled rate (1.25 lb a.i./acre). Although not often used and applied to small areas, there were a few instances where the average annual application rate for a crop use exceeded the maximum labeled rate. For example, the average application rate for Christmas tree (4.24 lb a.i./acre) was 6% greater than the maximum labeled rate (4.0 lb a.i./acre); however, only about 4.24 lb a.i./year was applied at this rate. There are a few limitations to the CDPR PUR data. There were several uses reported in the PUR data that were either not registered or were misuses according to labels; these accounted for approximately 5,400 lb a.i./year.

For almost all of the reported crops and uses in the CDPR PUR data, the 95th and 99th percentile estimations of application rates are also well below the maximum labeled rates. Again, the few exceptions to this likely occurred due to a few applications made to small areas. Only the maximum label application rates were modeled for this assessment.

Evaluation of the usage data (aggregated general cropping category) showed that 2,4-D was applied in the greatest quantity to cereal grains with an average annual application of ~150,600 lb a.i./year. The nuts, right of ways, pasture/grassland, pome/stone fruits, and turf cropping categories followed with ~85,900 lb a.i./year, ~60,400 lb a.i./year, ~43,900 lb a.i./year, ~24,500 lb a.i./year, and ~19,300 lb a.i./year, respectively.

A summary of all 2,4-D uses based on general cropping categories in California is provided in **Table 2.5**, and county use data is provided in **Table 2.6**. More detailed cropping use and acreage information based on specific PUR crop categories is available in **Appendix C**.

Table 2.5 Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) 2,4-D Use Data from 1999 to 2006

General Cropping Category	Average Annual Application (lb a.i./year)	Application Rate (lb a.i./acre)			
		AVG	95th %ile	99 %ile	MAX
Cereal Grains, Grain or Forage Sorghum	150,631	0.84	1.34	1.61	61.87
Nut Orchards, Pistachios, Filberts	85,903	0.60	0.91	1.20	27.03
Right of way, landscape maintenance	60,362	1.66	2.60	3.40	82.08
Grass Pastures, rangeland	43,924	1.10	2.09	2.70	29.41
Pome Fruits, Stone Fruits	24,524	0.489	0.75	0.93	15.50
Grass Pastures, rangeland, grass Grown for Seed and Sod, Ornamental Turf	19,322	0.95	1.28	1.45	44.78
Field Corn, Popcorn, Sweet Corn, Sunflower	13,677	0.64	0.94	1.03	10.26
Grapes	13,520	0.65	1.38	1.61	10.04
Citrus	13,155	0.28	0.40	0.44	22.00
Forestry, Tree and Brush Control, Christmas trees	7,987	3.00	4.35	4.88	42.41
Rice	7,827	0.45	0.76	1.13	5.43
Structural pest control, industrial sites	937	1.20	1.74	1.74	2.40

Table 2.5 Summary of California Department of Pesticide Registration (CDPR) Pesticide Use Reporting (PUR) 2,4-D Use Data from 1999 to 2006					
General Cropping Category	**Average Annual Application (lb a.i./year)**	**Application Rate (lb a.i./acre)**			
		AVG	**95th %ile**	**99 %ile**	**MAX**
Water area	374	3.62	3.77	4.46	13.80
Asparagus, dried bean, fruiting pepper, fallow land	310	0.63	0.70	0.70	1.72
Potato	42	0.64	0.79	0.79	1.13
Sugar beet, sugar cane	37	1.02	1.17	1.17	1.72
Reported PUR data, uses not represented on Master Label	9870	1.12	3.10	4.91	19.70
Uses Without PRZM Modeling Scenarios	30,153	1.78	2.49	2.50	61.08
TOTAL	**482,560**				

Table 2.6 Summary of California Department of Pesticide Registration (CDPR) Pesticide Usage Reporting (PUR) Data from 1999 to 2006 for Counties					
County	**Average Annual Application (lb a.i./year)**	**Application Rate (lb a.i./acre)**			
		Average	**95th %ile**	**99 %ile**	**Maximum**
SAN JOAQUIN	40,352	0.803	1.151	1.682	61.866
MERCED	33,696	0.776	1.178	1.378	9.010
KINGS	33,097	0.778	1.115	1.150	9.475
IMPERIAL	32,975	1.030	1.378	1.392	8.079
SOLANO	28,329	0.806	1.225	2.115	16.352
FRESNO	28,092	0.654	1.159	1.304	10.037
YOLO	26,369	0.896	1.138	1.940	27.029
STANISLAUS	23,870	0.910	1.319	1.545	14.250
TULARE	23,598	0.566	0.925	1.590	22.000
GLENN	19,361	0.692	1.070	1.373	15.754
BUTTE	18,9645	0.759	1.141	1.291	8.478
SACRAMENTO	16,657	1.228	1.472	1.785	13.800
MADERA	14,745	0.800	1.261	1.394	16.369
SISKIYOU	13,378	1.249	3.023	3.162	44.780
SUTTER	11,605	0.627	0.954	1.150	5.626
RIVERSIDE	11,164	1.571	2.760	3.066	82.075
MODOC	9,244	0.834	1.290	1.335	10.278
KERN	9,162	1.293	2.882	2.986	61.077
SAN LUIS OBISPO	8,983	0.567	0.864	1.703	22.611
COLUSA	8,214	0.545	0.853	0.987	5.626
ALAMEDA	7,197	1.477	3.731	5.308	31.655
CONTRA COSTA	6,445	1.223	2.071	2.176	9.002
TEHAMA	6,146	0.788	1.229	1.469	15.504
MONTEREY	5,061	0.860	1.228	1.492	4.524
LOS ANGELES	5,029	1.854	3.384	3.480	19.000

Table 2.6 Summary of California Department of Pesticide Registration (CDPR) Pesticide Usage Reporting (PUR) Data from 1999 to 2006 for Counties

SHASTA	4,031	1.033	1.374	1.886	7.727
SAN BERNARDINO	3,107	0.967	1.155	1.266	4.341
SANTA BARBARA	3,000	0.833	1.240	3.080	26.257
SAN BENITO	2,830	0.917	1.344	1.397	7.648
ORANGE	2,817	1.413	2.313	2.313	13.480
VENTURA	2,726	0.960	1.554	2.969	40.718
LASSEN	2,689	1.076	1.730	2.136	17.364
SANTA CLARA	2,340	0.834	1.388	1.914	9.892
SONOMA	2,074	0.988	1.430	1.602	9.501
YUBA	1,762	0.700	1.088	1.182	3.291
SAN DIEGO	1,755	0.564	1.109	1.168	13.346
PLACER	1,288	1.030	1.899	1.975	13.573
DEL NORTE	1,124	0.977	0.996	0.996	11.354
SAN MATEO	1,084	2.282	4.040	8.112	29.407
LAKE	951	1.087	1.524	3.027	16.879
AMADOR	937	0.931	1.232	1.358	5.652
MARIN	858	1.483	2.131	2.211	6.773
HUMBOLDT	792	0.818	1.465	1.712	6.362
TUOLUMNE	653	0.701	1.558	1.692	6.873
CALAVERAS	553	1.226	2.957	3.815	13.567
SIERRA	541	1.111	1.418	1.465	5.652
MENDOCINO	525	1.619	5.315	5.316	42.413
PLUMAS	522	0.994	1.329	1.406	3.815
NEVADA	324	1.162	2.143	2.143	5.805
EL DORADO	281	1.406	2.150	2.150	6.783
INYO	273	1.786	2.964	2.964	4.505
NAPA	263	0.586	0.924	0.924	1.697
MONO	255	1.968	2.005	2.005	4.076
TRINITY	244	1.771	5.829	6.318	13.188
SAN FRANCISCO	117	NR	NR	NR	NR
MARIPOSA	70	0.979	1.464	1.592	2.287
SANTA CRUZ	31	0.702	0.751	0.986	2.110
ALPINE	3	2.828	2.828	2.828	2.828
TOTAL	**482,560**				
NR – Not Reported					

2.5 Assessed Species

Table 2.7 provides a summary of the current distribution, habitat requirements, and life history parameters for the two listed species being assessed. More detailed life history and distribution information can be found in **Attachments 1** and **3**. See **Figures 2.4.a** and **2.4.b** for maps of the current range and designated critical habitat of the assessed listed species.

Table 2.7. Summary of Current Distribution, Habitat Requirements, and Life History Information for the Assessed Listed Species[1]						
Assessed Species	**Size**	**Current Range**	**Habitat Type**	**Designated Critical Habitat?**	**Reproductive Cycle**	**Diet**
California red-legged frog (*Rana aurora draytonii*)	Adult (85-138 cm in length), Females – 9-238 g, Males – 13-163 g; Juveniles (40-84 cm in length)	Northern CA coast, northern Transverse Ranges, foothills of Sierra Nevada, and in southern CA south of Santa Barbara	Freshwater perennial or near-perennial aquatic habitat with dense vegetation; artificial impoundments; riparian and upland areas	Yes	Breeding: Nov. to Apr. Tadpoles: Dec. to Mar. Young juveniles: Mar. to Sept.	Aquatic-phase[2]: algae, freshwater aquatic invertebrates Terrestrial-phase: aquatic and terrestrial invertebrates, small mammals, fish and frogs
AW (*Masticophis lateralis euryxanthus*)	3 – 5 ft	Contra Costa and Alameda Counties in California (additional occurrences in San Joaquin and Santa Clara Counties)	Primarily, scrub and chaparral communities. Also found in grassland, oak savanna, oak-bay woodland, and riparian areas.	Yes	Emerge from hibernation and begin mating from late March through mid-June. Females lay eggs in May through July. Eggs hatch from August through November. Hibernate during the winter months.	Lizards, small mammals, nesting birds, other snakes including rattlesnakes

[1]For more detailed information on the distribution, habitat requirements, and life history information of the assessed listed species, see Attachments 1 and 3
[2]For the purposes of this assessment, tadpoles and submerged adult frogs are considered "aquatic" because exposure pathways in the water are considerably different than those that occur on land.
[3]Oviparous = eggs hatch within the female's body and young are born live.

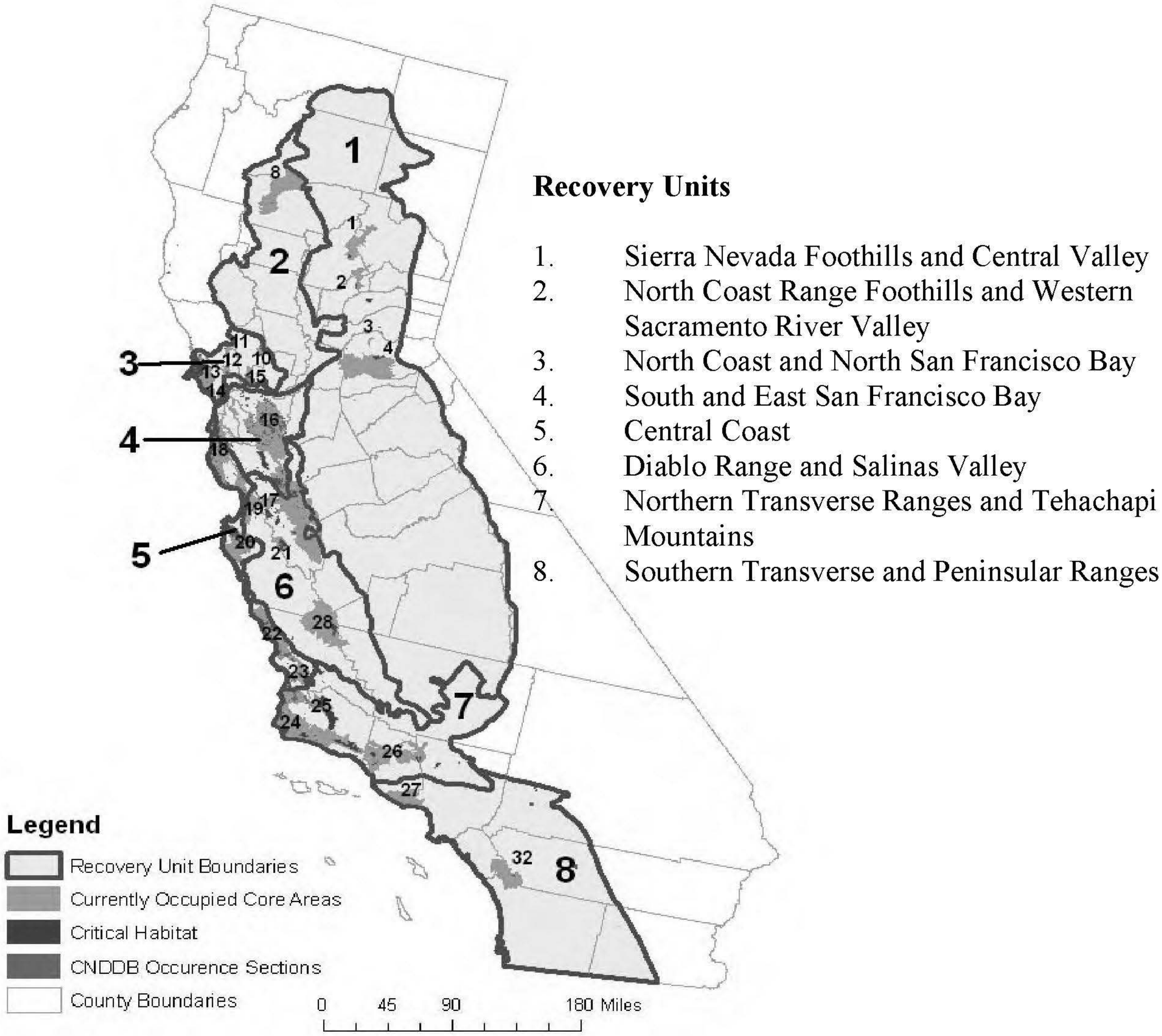


Figure 2.4.a Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF

Core Areas

1. Feather River
2. Yuba River- S. Fork Feather River
3. Traverse Creek/ Middle Fork/ American R. Rubicon
4. Cosumnes River
5. South Fork Calaveras River*
6. Tuolumne River*
7. Piney Creek*
8. Cottonwood Creek
9. Putah Creek – Cache Creek*
10. Lake Berryessa Tributaries
11. Upper Sonoma Creek
12. Petaluma Creek – Sonoma Creek
13. Pt. Reyes Peninsula
14. Belvedere Lagoon
15. Jameson Canyon – Lower Napa River
16. East San Francisco Bay
17. Santa Clara Valley
18. South San Francisco Bay
19. Watsonville Slough-Elkhorn Slough
20. Carmel River – Santa Lucia
21. Gablan Range
22. Estero Bay
23. Arroyo Grange River
24. Santa Maria River – Santa Ynez River
25. Sisquoc River
26. Ventura River – Santa Clara River
27. Santa Monica Bay – Venura Coastal Streams
28. Estrella River
29. San Gabriel Mountain*
30. Forks of the Mojave*
31. Santa Ana Mountain*
32. Santa Rosa Plateau
33. San Luis Ray*
34. Sweetwater*
35. Laguna Mountain*

* Core areas that were historically occupied by the California red-legged frog are not included in the map.

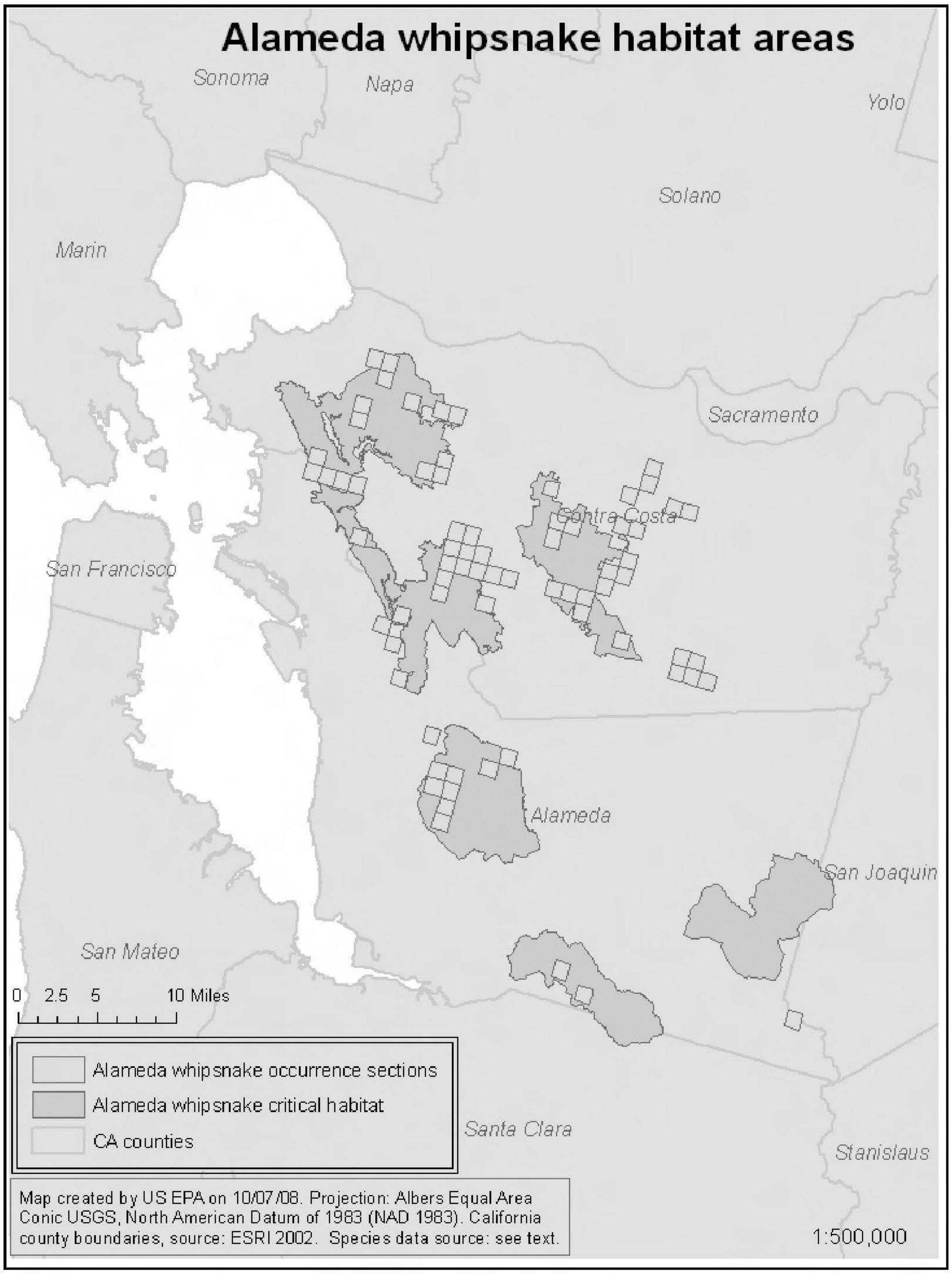


Figure 2.4.b Critical Habitat and Occurrence Designations for AW

2.6 Designated Critical Habitat

Critical habitats have been designated for the CRLF and AW.

'Critical habitat' is defined in the ESA as the geographic area occupied by the species at the time of the listing where the physical and biological features necessary for the conservation of the species exist, and there is a need for special management to protect the listed species. It may also include areas outside the occupied area at the time of listing if such areas are 'essential to the conservation of the species.' Critical habitat receives protection under Section 7 of the ESA through prohibition against destruction or adverse modification with regard to actions carried out, funded, or authorized by a federal agency. Section 7 requires consultation on federal actions that are likely to result in the destruction or adverse modification of critical habitat.

To be included in a critical habitat designation, the habitat must be 'essential to the conservation of the species.' Critical habitat designations identify, to the extent known using the best scientific and commercial data available, habitat areas that provide essential life cycle needs of the species, or areas that contain certain primary constituent elements (PCEs) (as defined in 50 CFR 414.12(b)). PCEs include, but are not limited to, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species. **Table 2.8** describes the PCEs for the critical habitats designated for the CRLF and AW.

Table 2.8 Designated Critical Habitat PCEs for the CRLF and AW[1]

Species	PCEs	Reference
CRLF	Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond.	50 CFR 414.12(b), 2006
	Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	
	Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	
	Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.,* algae)	
	Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	
	Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	

Table 2.8 Designated Critical Habitat PCEs for the CRLF and AW[1]		
Species	PCEs	Reference
	Reduction and/or modification of food sources for terrestrial phase juveniles and adults	
	Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	
AW	Scrub/shrub communities with a mosaic of open and closed canopy	71 FR 58175 58231, 2006
	Woodland or annual grassland plant communities contiguous to lands containing scrub/shrub communities with a mosaic of open and closed canopy	
	Lands containing rock outcrops, talus, and small mammal burrows within or adjacent to 1) scrub/shrub communities with a mosaic of open and closed canopy and/or 2) woodland or annual grassland plant communities contiguous to lands containing scrub/shrub communities with a mosaic of open and closed canopy	
[1] These PCEs are in addition to more general requirements for habitat areas that provide essential life cycle needs of the species such as, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species.		

More detail on the designated critical habitat applicable to this assessment can be found in **Attachment 1** (CRLF) and **Attachment 3** (AW). Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of 2,4-D that may alter the PCEs of the designated critical habitats for the CRLF and AW form the basis of the critical habitat impact analysis.

As previously noted in **Section 2.1**, the Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitats. Because 2,4-D is expected to directly impact living organisms within the action area, critical habitat analysis for 2,4-D is limited in a practical sense to those PCEs of critical habitats that are biological or that can be reasonably linked to biologically mediated processes.

2.7 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the federal action and not merely the immediate area involved in the action (50 CFR 402.02). It is recognized that the overall action area for the national registration of 2,4-D is likely to encompass considerable portions of the United States based on the large array of agricultural and/or non-agricultural uses. However, the scope of this assessment limits consideration of the overall action area to those portions that may be applicable to the protection of the CRLF and AW and their designated critical habitats within the state of California. Although the watershed for the San Francisco Bay extends northward into the very southwestern portion of Lake County, Oregon, and westward into the western edge of Washoe County, Nevada, the non-California portions of the watershed are small and very rural with little, if any, agriculture. Therefore, no use of 2,4-D is expected in these areas, and they are not considered as part of the action area applicable to this assessment.

The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled uses for 2,4-D. An analysis of labeled uses and review of available product labels was completed. This analysis was based on the Master Label (**Appendix N**) as supported by the 2,4-D Industry and the Interregional Research Project Number 4 (IR-4). Several of the currently labeled uses are special local needs (SLN) uses or are restricted to specific states other than California and are excluded from this assessment. In addition, a distinction has been made between food use crops and those that are non-food/non-agricultural uses. Uses relevant to the assessed species ans which constitute the federal action are listed in **Table 2.4**.

Following a determination of the assessed uses, the potential "footprint" of 2,4-D use patterns (*i.e.*, the area where pesticide application occurs) is determined. This "footprint" represents the initial area of concern, based on an analysis of available land cover data for the state of California. Deriving the geographical extent of this portion of the action area is based on consideration of the types of effects that 2,4-D may be expected to have on the environment, the exposure levels to 2,4-D that are associated with those effects, and the best available information concerning the use of 2,4-D and its fate and transport within the state of California. Specific measures of ecological effect for the CRLF and AW that define the action area include any direct and indirect toxic effect to the CRLF and AW and any potential modification of its critical habitat, including reduction in survival, growth, and fecundity as well as the full suite of 2,4-D effects available in the effects literature. Therefore, the action area extends to a point where environmental exposures are below any measured lethal or 2,4-D effect threshold for any biological entity at the whole organism, organ, tissue, and cellular level of organization. In situations where it is not possible to determine the threshold for an observed effect, the action area is not spatially limited and is assumed to be the entire state of California. Based on the broad range of 2,4-D use patterns, the large geographic coverage of those uses, as well as the large total poundage used, the entire state of California is considered to be the initial area of concern for this assessment.

Once the initial area of concern is defined, the next step is to define the potential boundaries of the action area by determining the extent of offsite transport via spray drift and runoff where exposure of one or more taxonomic groups to the pesticide exceeds the listed species LOCs.

The Agency's approach to defining the action area under the provisions of the Overview Document (U.S. EPA, 2004) considers the results of the risk assessment process to establish boundaries for that action area with the understanding that exposures below the Agency's defined Levels of Concern (LOCs) constitute a no-effect threshold. Deriving the geographical extent of this portion of the action area is based on consideration of the types of effects that 2,4-D may be expected to have on the environment, the exposure levels to 2,4-D that are associated with those effects, and the best available information concerning the use of 2,4-D and its fate and transport within the state of California. Specific measures of ecological effect for the assessed species that define the action area include any direct and indirect toxic effect to the assessed species and any potential modification of its critical habitat, including reduction in survival, growth, and fecundity as well as the full suite of sublethal effects available in the effects literature. Therefore, the action area extends to a point where environmental exposures are below any measured lethal or sublethal effect threshold for any biological entity at the whole organism, organ, tissue, and cellular level of organization. In situations where it is not possible to determine the threshold for an observed effect, the action area is not spatially limited and is assumed to be the entire state of California.

Due to the lack of a defined no effect concentration in a guideline terrestrial plant study (vegetative vigor, tomato and turnip NOAEC < 0.00134 lb a.e./acre for dry weight, MRID 471060-04), the spatial extent of the action area (*i.e.*, the boundary where exposures and potential effects are less than the Agency's LOC) for 2,4-D cannot be determined. Therefore, it is assumed that the action area encompasses the entire state of California, regardless of the spatial extent (*i.e.*, initial area of concern or footprint) of the pesticide use(s).

2.8 Assessment Endpoints and Measures of Ecological Effect

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[4] Selection of the assessment endpoints is based on valued entities (*e.g.,* CRLF and AW), organisms that are important in the life cycle of the assessed species, and the PCEs of its designated critical habitat), the ecosystems potentially at risk (*e.g.,* water bodies, riparian vegetation, and upland and dispersal habitats), the migration pathways of 2,4-D (*e.g.*, runoff, spray drift, *etc*.), and the routes by which ecological receptors are exposed to 2,4-D *(e.g.,* direct contact, *etc.).*

2.8.1 Bridging Strategy for Toxicological Data

EFED established a strategy for ecological toxicity studies submitted in support of 2,4-D and its formulations. In this document, the term formulation is used to refer to the 2,4-D Task Force supported technical formulations listed below, while the term end use product is used to refer to any formulated product including mixtures of pesticide sold in the US. All toxicity values have been converted to the acid equivalent (a.e.) based on the ratio of molecular weights.

For aquatic animals (including aquatic phase amphibians) and plants, data evaluating 2,4-D acid and salts have been bridged, while the data evaluating the three esters was separately bridged. On an a.e. basis, toxicity to the acid and salts is comparable; however, toxicity to the esters tends to be two to three orders of magnitude higher. In addition, fate data were submitted suggesting that the salts dissociate rapidly to the acid, on the order of several minutes. However the esters may take longer to hydrolyze to the acid, especially depending on pH of the water. For terrestrial animals (including terrestrial phase

[4] From U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.

amphibians) and plants, data evaluating 2,4-D acid, salts and esters have been bridged. Within an organism group, the variation in the toxicity endpoints is less than two orders of magnitude, and for some groups, the variation is less than one order of magnitude. Within each of these bridged groups of 2,4-D formulations, the most sensitive toxicity endpoint was used for risk estimation. Toxicity data were not available for all taxa and all formulations. In those cases it was assumed that toxicity would be similar as in the other formulations in the same group. **Table 2.9** summarizes 2,4-D bridging strategies for estimating acute and chronic toxicity to aquatic and terrestrial organisms and plants.

Table 2.9 Summary of toxicity bridging strategies for 2,4-D	
Acid and Salts bridged for estimating acute toxicity to aquatic organisms and plants[a]	
PC Code	**Chemical Name**
030001	2,4D acid
030004	2,4D sodium salt
030016	2,4D diethanolamine (DEA) salt
030019	2,4D dimethylamine (DMA) salt
030025	2,4D Isoproylamine (IPA) salt
030035	2,4D triisopropanolamine (TIPA) salt
Esters bridged for estimating acute toxicity to aquatic organisms and plants	
PC Code	**Chemical Name**
030053	2,4D butoxyethyl (BEE) ester
030063	2,4D 2 ethylhexyl ester (EHE)
030066	2,4D isopropyl ester (IPE)
Acid, Salts, and Esters bridged for estimating acute and chronic toxicity to terrestrial organisms and plants	
PC Code	**Chemical Name**
030001	2,4D acid
030004	2,4D sodium salt
030016	2,4D diethanolamine (DEA) salt
030019	2,4D dimethylamine (DMA) salt
030025	2,4D Isoproylamine (IPA) salt
030035	2,4D triisopropanolamine (TIPA) salt
030053	2,4D butoxyethyl (BEE) ester
030063	2,4D 2 ethylhexyl ester (EHE)
030066	2,4D isopropyl ester (IPE)
[a]For aquatic organisms, chronic toxicity data from acid and salts also used for chronic toxicity to esters, as long-term exposure to the esters was not expected.	

2.8.2 Assessment Endpoints

Assessment endpoints for the CRLF and AW include direct toxic effects on the survival, reproduction, and growth of individuals, as well as indirect effects, such as reduction of the prey base or modification of their habitats. In addition, potential modification of critical habitat is assessed by evaluating potential effects to PCEs, which are components of the habitat areas that provide essential life cycle needs of the assessed species. Each assessment endpoint requires one or more "measures of ecological effect," defined as changes in the attributes of an assessment endpoint or changes in a surrogate entity or attribute in response to exposure to a pesticide. Specific measures of ecological effect are generally evaluated based on acute and chronic toxicity information from registrant-submitted guideline tests that are performed on a limited number of organisms. Additional ecological effects data from the open literature are also considered. It should be noted that assessment endpoints are limited to direct and indirect effects associated with survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According to the Overview Document (U.S. EPA, 2004), the Agency relies on acute and chronic effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer-reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

A discussion of all the toxicity data available for this risk assessment, including resulting measures of ecological effect selected for each taxonomic group of concern, is included in **Section 4** of this document. A summary of the assessment endpoints and measures of ecological effect selected to characterize potential assessed direct and indirect risks for each of the assessed species associated with exposure to 2,4-D is provided in **Section 2.5** and **Table 2.10**.

As described in the Agency's Overview Document (U.S. EPA, 2004), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include aquatic-phase amphibians, freshwater fish, freshwater invertebrates, aquatic plants, birds (surrogate for terrestrial-phase amphibians), mammals, terrestrial invertebrates, and terrestrial plants. Acute (short-term) and chronic (long-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on 2,4-D.

Table 2.10 identifies the taxa used to assess the potential for direct and indirect effects from the uses of 2,4-D for each listed species assessed here. The specific assessment endpoints used to assess the potential for direct and indirect effects to each listed species are provided in **Table 2.11**.

Table 2.10. Taxa Used in the Analyses of Direct and Indirect Effects for the Assessed Listed Species							
Listed Species	**Birds[1]**	**Mammals**	**Terrestrial Plants**	**Terrestrial Inverts.**	**Freshwater Fish[2]**	**Freshwater Inverts.**	**Aquatic Plants**
CRLF	Direct Indirect (prey)	Indirect (prey)	Indirect (habitat)	Indirect (prey)	Direct Indirect (prey)	Indirect (prey)	Indirect (food/ habitat)
AW	Direct Indirect (prey)	Indirect (prey)	Indirect (habitat)	Indirect (prey)	N/A	N/A	N/A

[1] Birds are used as surrogates for the terrestrial-phase CRLF and for the AW.
[2] Fish are used as surrogates for the aquatic-phase CRLF.
N/A = Not applicable

Table 2.11 Taxa and Assessment Endpoints Used to Evaluate the Potential for the Use of 2,4-D to Result in Direct and Indirect Effects to the CRLF and the AW			
Taxa Used to Assess Direct and/or Indirect Effects to Assessed Species	**Assessed Listed Species**	**Assessment Endpoints**	**Measures of Ecological Effects[1]**
1. Freshwater Fish and Aquatic-phase Amphibians	<u>Direct Effect</u> Aquatic-phase CRLF	Survival, growth, and reproduction of individuals via direct effects	**Acid/Salts** 1a. Common carp acute LC_{50} 1b. Fathead minnow chronic NOAEC **Esters** 1c. Bluegill sunfish acute LC_{50}
	<u>Indirect Effect (prey)</u> Aquatic-phase and Terrestrial-phase CRLF	Survival, growth, and reproduction of individuals via indirect effects on aquatic prey food supply (*i.e.*, fish and aquatic-phase amphibians)	
2. Freshwater Invertebrates	<u>Indirect Effect (prey)</u> Aquatic-phase and Terrestrial-phase CRLF	Survival, growth, and reproduction of individuals via indirect effects on aquatic prey food supply (*i.e.*, freshwater invertebrates)	**Acid/Salts** 2a. Daphnid acute EC_{50} 2b. Daphnid chronic NOAEC **Esters** 2c. Daphnid acute EC_{50}
3. Aquatic Plants (freshwater)	<u>Indirect Effect (food/habitat)</u> Aquatic-phase CRLF	Survival, growth, and reproduction of individuals via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.*, aquatic plant community)	**Acid/Salts** 5a. Water Milfoil EC_{50} (vascular plant) 5b. *Navicula pelliculosa* EC_{50} (freshwater diatom) **Esters** 5c. Duckweed EC_{50} (vascular plant) 5d. *Skeletonema costatum* EC_{50} (marine diatom)
4. Birds	<u>Direct Effect</u> Terrestrial-phase CRLF AW	Survival, growth, and reproduction of individuals via direct effects	**Acid/Salts/Esters** 6a. Bobwhite quail gavage acute LD_{50} & Bobwhite quail and mallard dietary acute LC_{50} 6b. Bobwhite quail chronic NOAEC

Table 2.11 Taxa and Assessment Endpoints Used to Evaluate the Potential for the Use of 2,4-D to Result in Direct and Indirect Effects to the CRLF and the AW			
Taxa Used to Assess Direct and/or Indirect Effects to Assessed Species	**Assessed Listed Species**	**Assessment Endpoints**	**Measures of Ecological Effects[1]**
	Indirect Effect (prey) Terrestrial-phase CRLF AW	Survival, growth, and reproduction of individuals via indirect effects on terrestrial prey (birds)	
5. Mammals	Indirect Effect (prey/habitat from burrows) Terrestrial-phase CRLF AW	Survival, growth, and reproduction of individuals via indirect effects on terrestrial prey (mammals)	**Acid/Salts/Esters** 7a. Laboratory rat acute LD_{50} 7b. Laboratory rat chronic NOAEC
6. Terrestrial Invertebrates	Indirect Effect (prey) Terrestrial-phase CRLF AW	Survival, growth, and reproduction of individuals via indirect effects on terrestrial prey (terrestrial invertebrates)	**Acid/Salts/Esters** 8a. Honey bee acute LD_{50}
7. Terrestrial Plants	Indirect Effect (food/habitat) (non-obligate relationship) Terrestrial-phase CRLF AW	Survival, growth, and reproduction of individuals via indirect effects on food and habitat (*i.e.*, riparian and upland vegetation)	**Acid/Salts/Esters** 9a. Monocot EC_{25}: onion (seedling emergence and vegetative vigor) 9b. Dicot $EC_{25:}$ tomato (seedling emergence) and lettuce (vegetative vigor)
[1] Toxicity data for the nine technical formulations of 2,4-D were bridged according to the taxonomic group, and the chemical composition (acid, salt, ester). The summaries here reflect this established bridging strategy. More background and details are found in **Section 1, Section 2.2, and Section 4.2.** The species listed is the most sensitive within each classification (acid/salts, esters, or acid/salts/esters).			

2.8.3 Assessment Endpoints for Designated Critical Habitat

As previously discussed, designated critical habitat is assessed to evaluate actions related to the use of 2,4-D that may alter the PCEs of the assessed species' designated critical habitats. PCEs for the assessed species were previously described in **Section 2.6**. Actions that may modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the assessed species. Therefore, these actions are identified as assessment endpoints. It should be noted that evaluation of PCEs as assessment endpoints is limited to those of a biological nature (*i.e.*, the biological resource requirements for the listed species associated with the critical habitat) and those for which 2,4-D effects data are available.

Some components of these PCEs are associated with physical abiotic features (*e.g.*, presence and/or depth of a water body, or distance between two sites), which are not expected to be measurably altered by use of pesticides. Measures of ecological effect used to assess the potential for adverse modification to the critical habitat of the CRLF and AW are described in **Table 2.12**.

Table 2.12 Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat for CRLF and AW			
Taxon Used to Assess Modification of PCE	**Assessed Listed Species Associated with the PCE**	**Assessment Endpoints**	**Measures of Ecological Effects**[1]
1. Freshwater Fish and Aquatic-phase Amphibians	Direct Effect Aquatic-phase CRLF	Survival, growth, and reproduction of individuals via direct effects	**Acid/Salts** 1a. Rainbow trout acute LC_{50} 1b. Fathead minnow chronic NOAEC **Esters** 1c. Bluegill sunfish acute LC_{50}
	Indirect Effect (prey) Aquatic-phase and Terrestrial-phase CRLF	Modification of critical habitat via change in aquatic prey food supply (*i.e.,* fish and aquatic-phase amphibians)	
2. Freshwater Invertebrates	Indirect Effect (prey) Aquatic-phase and Terrestrial-phase CRLF	Survival, growth, and reproduction of individuals via indirect effects on aquatic prey food supply (*i.e.,* freshwater invertebrates)	**Acid/Salts** 2a. Daphnid acute EC_{50} 2b. Daphnid chronic NOAEC **Esters** 2c. Daphnid acute EC_{50}
3. Aquatic Plants (freshwater)	Indirect Effect (food/habitat) Aquatic-phase CRLF	Modification of critical habitat via change in habitat, cover, food supply, and/or primary productivity (*i.e.,* aquatic plant community)	**Acid/Salts** 3a. Water Milfoil EC_{50} (vascular plant) 3b. *Navicula pelliculosa* EC_{50} (freshwater diatom) **Esters** 3c. Duckweed EC_{50} (vascular plant) 3d. *Skeletonema costatum* EC_{50} (marine diatom)
4. Birds	Direct Effect Terrestrial-phase CRLF AW	Survival, growth, and reproduction of individuals via direct effects	**Acid/Salts/Esters** 4a. Bobwhite quail gavage acute LD_{50} & Bobwhite quail and mallard dietary acute LC_{50} 4b. Bobwhite quail chronic NOAEC
	Indirect Effect (prey) Terrestrial-phase CRLF AW	Modification of critical habitat via change in terrestrial prey (birds)	
5. Mammals	Indirect Effect (prey/habitat from burrows) Terrestrial-phase CRLF AW	Modification of critical habitat via change in terrestrial prey (mammals)	**Acid/Salts/Esters** 5a. Laboratory rat acute LD_{50} 5b. Laboratory rat chronic NOAEC
6. Terrestrial Invertebrates	Indirect Effect (prey) Terrestrial-phase CRLF AW	Modification of critical habitat via change in terrestrial prey (terrestrial invertebrates)	**Acid/Salts/Esters** 6a. Honey bee acute LD_{50}

Table 2.12 Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat for CRLF and AW			
Taxon Used to Assess Modification of PCE	**Assessed Listed Species Associated with the PCE**	**Assessment Endpoints**	**Measures of Ecological Effects[1]**
7. Terrestrial Plants	Indirect Effect (food/habitat) (non-obligate relationship) Terrestrial-phase CRLF AW	Modification of critical habitat via change in food and habitat (*i.e.*, riparian and upland vegetation)	**Acid/Salts/Esters** 7a. Monocot EC_{25}: onion (seedling emergence and vegetative vigor) 7b. Dicot $EC_{25:}$ tomato (seedling emergence) and lettuce (vegetative vigor)
[1]Toxicity data for the nine technical formulations of 2,4-D were bridged according to the taxonomic group, and the chemical composition (acid, salt, ester). The summaries here reflect this established bridging strategy. More background and details are found in **Section 1, Section 2.2,** and **Section 4.2.** The species listed is the most sensitive within each classification (acid/salts, esters, or acid/salts/esters).			

2.9 Conceptual Model

2.9.1 Risk Hypotheses

Risk hypotheses are specific assumptions about potential adverse effects (*i.e.,* changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical models, or probability models (U.S. EPA, 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of 2,4-D to the environment. The following risk hypotheses are presumed for each assessed species in this assessment:

The labeled use of 2,4-D within the action area may:

- directly affect the CRLF and/or AW by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect the CRLF and/or AW by modifying the designated critical habitat by reducing or changing the composition of food supply;
- indirectly affect the CRLF by modifying the designated critical habitat by reducing or changing the composition of the aquatic plant community in the species' current range, thus affecting primary productivity and/or cover;
- indirectly affect the CRLF and/or AW by modifying the designated critical habitat by reducing or changing the composition of the terrestrial plant community in the species' current range;
- indirectly affect the CRLF by modifying the designated critical habitat by reducing or changing aquatic habitat in their current range (via modification of water quality parameters, habitat morphology, and/or sedimentation);
- modify the designated critical habitat of the CRLF by reducing or changing upland habitat within 200 ft of the edge of the riparian vegetation necessary for shelter, foraging, and predator avoidance;

- modify the designated critical habitat of the CRLF by reducing or changing dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal.

2.9.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the 2,4-D release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for aquatic and terrestrial phases of the CRLF and AW and the conceptual models for the aquatic and terrestrial PCE components of critical habitats are shown in **Figures 2.5** and **2.6**. Although the conceptual models for direct/indirect effects and modification of designated critical habitat PCEs are shown on the same diagrams, the potential for direct/indirect effects and modification of PCEs will be evaluated separately in this assessment. Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those potential exposure routes to potential risks to the CRLF and AW and modification to designated critical habitats is expected to be negligible.

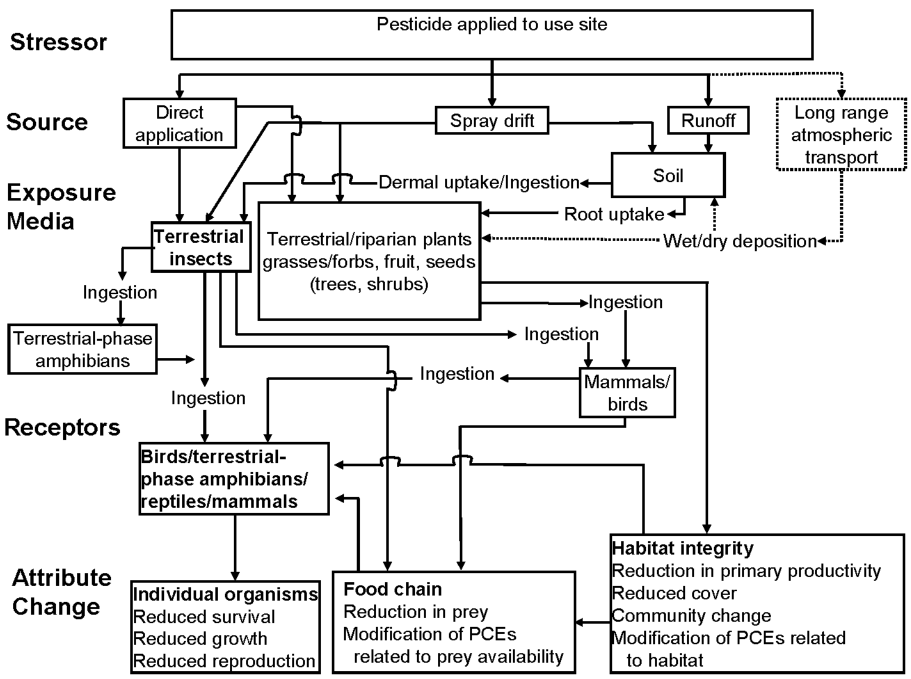


Figure 2.5 Conceptual Model for Terrestrial-Phase of the CRLF and AW (applicable to the acid, salt, and ester technical formulations of 2,4-D)

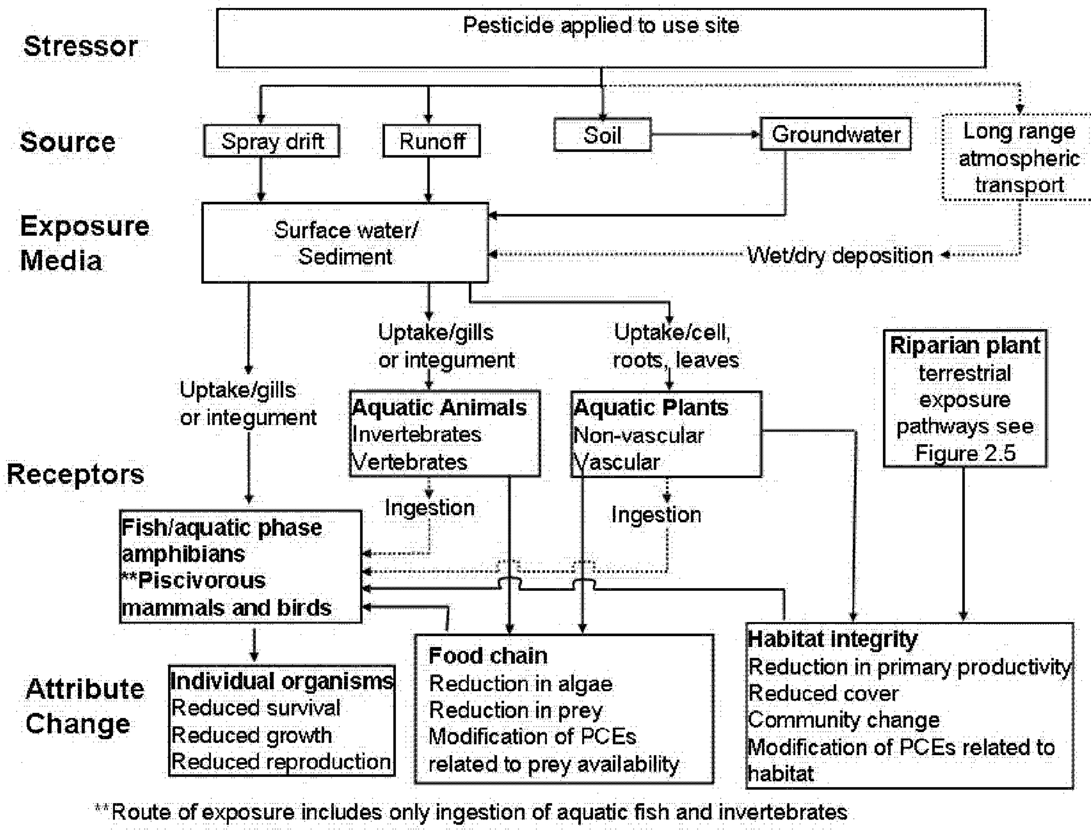


Figure 2.6a Conceptual Model for Aquatic-Phase of the CRLF (applicable to the acid and salt technical formulations of 2,4-D, also applicable to ester technical forms of 2,4-D for acute exposure (assuming all the ester has hydrolyzed to the acid prior to reaching the water body) and for chronic exposure.

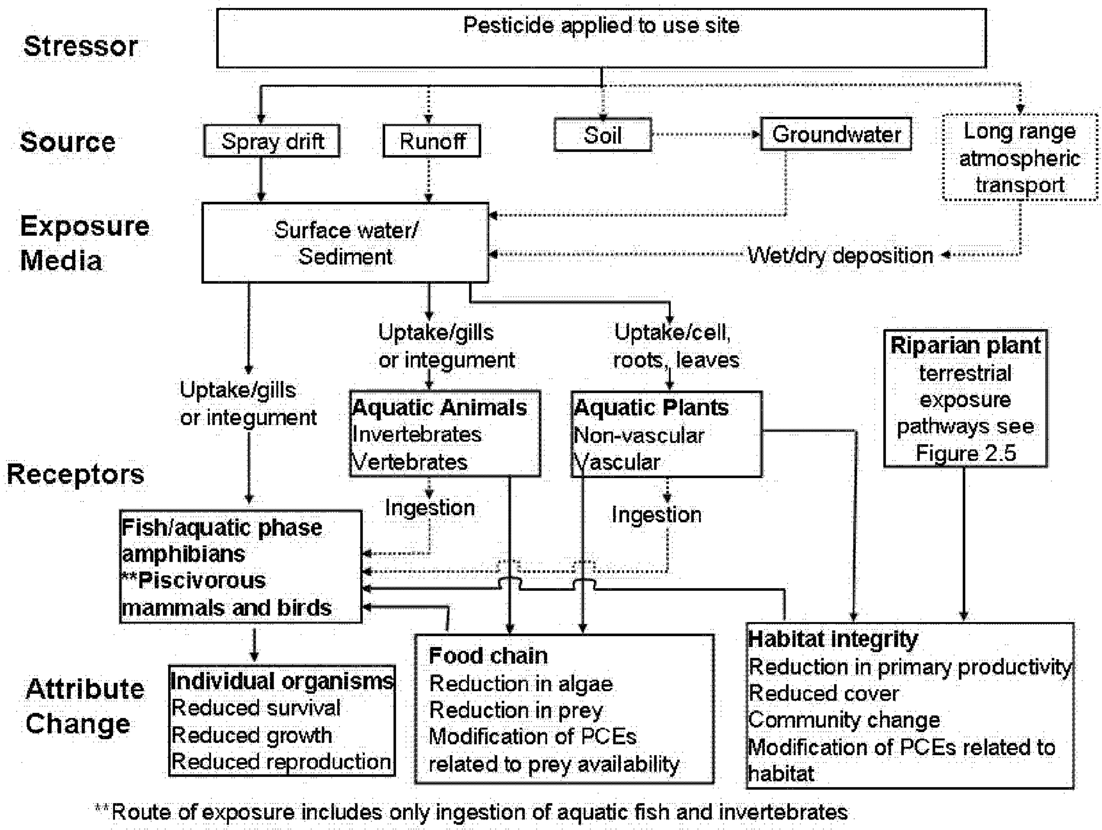


Figure 2.6b Conceptual Model for Aquatic-Phase of the CRLF (applicable to the ester technical formulations of 2,4-D, assuming ester has not yet hydrolyzed at time of exposure).

2.10 Analysis Plan

In order to address the risk hypothesis, the potential for direct and indirect effects to the CRLF and AW, prey items, and habitat is estimated based on a taxon-level approach. In the following sections, the use, environmental fate, and ecological effects of 2,4-D are characterized and integrated to assess the risks. This is accomplished using a risk quotient (ratio of exposure concentration to effects concentration) approach. Although risk is often defined as the likelihood and magnitude of adverse ecological effects, the risk quotient-based approach does not provide a quantitative estimate of likelihood and/or magnitude of an adverse effect. However, as outlined in the Overview Document (U.S. EPA, 2004), the likelihood of effects to individual organisms from particular uses of 2,4-D is estimated using the probit dose-response slope and either the level of concern (discussed below) or actual calculated risk quotient value.

2.10.1 Measures to Evaluate the Risk Hypothesis and Conceptual Model

2.10.1.1 Measures of Exposure

The environmental fate properties of 2,4-D along with available monitoring data indicate that runoff and spray drift are the principle potential transport mechanisms of 2,4-D to the aquatic and terrestrial habitats of the CRLF and AW. In this assessment, transport of 2,4-D through runoff and spray drift is considered in deriving quantitative estimates of 2,4-D exposure to CRLF and AW, their prey, and their habitats.

Measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) of 2,4-D using maximum labeled application rates and methods of application. The models used to predict aquatic EECs are the Pesticide Root Zone Model coupled with the Exposure Analysis Model System (PRZM/EXAMS). The model used to predict terrestrial EECs on food items is T-REX. The model used to derive EECs relevant to terrestrial and wetland plants is TerrPlant. These models are parameterized using relevant reviewed registrant-submitted environmental fate data.

PRZM (v3.12.2, May 2005) and EXAMS (v2.98.4.6, April 2005) are screening simulation models coupled with the input shell pe5.pl (August 2007) to generate daily exposures and 1-in-10 year EECs of 2,4-D that may occur in surface water bodies adjacent to application sites receiving 2,4-D through runoff and spray drift. PRZM simulates pesticide application, movement, and transformation on an agricultural field and the resultant pesticide loadings to a receiving water body via runoff, erosion, and spray drift. EXAMS simulates the fate of the pesticide and resulting concentrations in the water body. The standard scenario used for ecological pesticide assessments assumes application to a 10-hectare agricultural field that drains into an adjacent 1-hectare water body, 2-meters deep (20,000 m^3 volume) with no outlet. PRZM/EXAMS was used to estimate screening-level exposure of aquatic organisms to 2,4-D. The measure of exposure for aquatic species is the 1-in-10 year return peak or rolling mean concentration. The 1-in-10-year 60-day mean is used for assessing chronic exposure to fish; the 1-in-10-year 21-day mean is used for assessing chronic exposure for aquatic invertebrates.

For the rice use, the Tier I rice model was used to estimate aquatic EECs. The model assumes partitioning of the pesticide between water and the upper 1 cm of sediment but does not include degradation. For the direct applications to water (*e.g.*, ditchbanks and water bodies), aquatic EECs were modeled using aerobic aquatic degradation rates.

Exposure estimates for the terrestrial animals assumed to be in the target area or in an area exposed to spray drift are derived using the T-REX model (version 1.4.1, October 8, 2008). This model incorporates the Kenega nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represented the 95th percentile of residue values from actual field measurements (Hoerger and Kenega, 1972).

For modeling purposes, direct exposures of the CRLF and AW to 2,4-D through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey (small mammals) are assessed using the small mammal (15 g) which consumes short grass. The small bird (20 g) consuming small insects and the small mammal (15 g) consuming short grass are used because these categories represent the largest RQs of the size and dietary categories in T-REX that are appropriate surrogates for the CRLF and AW and one of their prey items. Estimated exposures of terrestrial insects to 2,4-D are bound by using the dietary based EECs for small insects and large insects.

Birds are currently used as surrogates for terrestrial-phase amphibians and reptiles. However, amphibians and reptiles are poikilotherms (body temperature varies with environmental temperature) while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, amphibians and reptiles tend to have much lower metabolic rates and lower caloric intake requirements than birds or mammals. As a consequence, birds are likely to consume more food than amphibians and reptiles on a daily dietary intake basis, assuming similar caloric content of the food items. Therefore, the use of avian food intake allometric equation as a surrogate to amphibians and reptiles is likely to result in an over-estimation of exposure and risk for reptiles and terrestrial-phase amphibians. Therefore, T-REX has been refined to the T-HERPS model (v. 1.0), which allows for an estimation of food intake for poikilotherms using the same basic procedure as T-REX to estimate avian food intake.

EECs for terrestrial plants inhabiting dry and wetland areas are derived using TerrPlant (version 1.2.2, December 26, 2006). This model uses estimates of pesticides in runoff and in spray drift to calculate EECs. EECs are based upon solubility, application rate and minimum incorporation depth.

The spray drift model, AgDRIFT is used to assess exposure to 2,4-D deposited on terrestrial habitats by spray drift. In addition to the buffered area from the spray drift analysis, the downstream extent of 2,4-D that exceeds the LOC for the effects determination is also considered.

2.10.1.2 Measures of Effect

Data identified in **Section 2.10** are used as measures of effect for direct and indirect effects to the CRLF and AW. Data were obtained from registrant-submitted studies or from literature studies identified by ECOTOX. The ECOTOXicology database (ECOTOX) was searched in order to provide more ecological effects data and in an attempt to bridge existing data gaps. ECOTOX is a source for locating single chemical toxicity data for aquatic life, terrestrial plants, and wildlife. ECOTOX was created and is maintained by the U.S. EPA, Office of Research and Development, and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division.

The assessment of risk for direct effects to the terrestrial-phase CRLF and AW makes the assumption that toxicity of 2,4-D to birds is similar to or less than the toxicity to terrestrial-phase amphibians and reptiles (this also applies to potential prey items). The same assumption is made for fish and aquatic-phase CRLF (again, this also applies to potential prey items).

The acute measures of effect used for animals in this screening level assessment are the LD_{50}, LC_{50}, and EC_{50}. LD stands for "Lethal Dose", and LD_{50} is the amount of a material, given all at once, that is estimated to cause the death of 50% of the test organisms. LC stands for "Lethal Concentration" and LC_{50} is the concentration of a chemical that is estimated to kill 50% of the test organisms. EC stands for "Effective Concentration" and the EC_{50} is the concentration of a chemical that is estimated to produce a specific effect in 50% of the test organisms. Endpoints for chronic measures of exposure for listed and non-listed animals are the NOAEL/NOAEC and NOEC. NOAEL stands for "No Observed-Adverse-Effect-Level" and refers to the highest tested dose of a substance that has been reported to have no harmful (adverse) effects on test organisms. The NOAEC (*i.e.*, "No-Observed-Adverse-Effect-Concentration") is the highest test concentration at which none of the observed effects were statistically different from the control. The NOEC is the No-Observed-Effects-Concentration. For non-listed plants, only acute exposures are assessed (*i.e.*, EC_{25} for terrestrial plants and EC_{50} for aquatic plants).

It is important to note that the measures of effect for direct and indirect effects to the assessed species and their designated critical habitat are associated with impacts to survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According the Overview Document (U.S. EPA, 2004), the Agency relies on effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

2.10.1.3 Integration of Exposure and Effects

Risk characterization is the integration of exposure and ecological effects characterization to determine the potential ecological risk from agricultural and non-agricultural uses of 2,4-D, and the likelihood of direct and indirect effects to CRLF and AW in aquatic and terrestrial habitats. The exposure and toxicity effects data are integrated in order to evaluate the risks of adverse ecological effects on non-target species. For the assessment of 2,4-D risks, the risk quotient (RQ) method is used to compare exposure and measured toxicity values. EECs are divided by acute and chronic toxicity values. The resulting RQs are then compared to the Agency's levels of concern (LOCs) (U.S. EPA, 2004) (see **Appendix I**).

For this endangered species assessment, listed species LOCs are used for comparing RQ values for acute and chronic exposures of 2,4-D directly to the CRLF and AW. If estimated direct exposures to the assessed species of 2,4-D resulting from a particular use are sufficient to exceed the listed species LOC, then the effects determination for that use is "may affect". When considering indirect effects to the assessed species due to effects to prey, the listed species LOCs are also used. If estimated exposures to the prey of the assessed species of 2,4-D resulting from a particular use are sufficient to exceed the listed species LOC, then the effects determination for that use is a "may affect." If the RQ being considered also exceeds the non-listed species acute risk LOC, then the effects determination is a LAA. If the acute RQ is between the listed species LOC and the non-listed acute risk species LOC, then further lines of evidence (*i.e.*, probability of individual effects, species sensitivity distributions) are considered in distinguishing between a determination of NLAA and a LAA. If the RQ being considered for a particular use exceeds the non-listed species LOC for plants, the effects determination is "may affect". Further information on LOCs is provided in **Appendix I**.

2.10.2 Data Gaps

2.10.2.1 Fate and Transport Data

The registrant-submitted fate and transport data (classified as either Acceptable or Supplemental) provide sufficient information for EFED to identify 2,4-D routes of dissipation in surface soils and water and, therefore, were sufficient to conduct the risk assessment. No apparent data gaps were identified in the fate and transport database. The summaries of environmental fate studies of 2,4-D are presented in **Appendix B**.

2.10.2.2 Ecotoxicity Data

The registrant-submitted ecotoxicity data and open literature ECOTOX data (classified as either Acceptable or Supplemental) provide sufficient information for EFED to identify 2,4-D routes of exposure to aquatic and terrestrial organisms. No apparent data gaps were identified in the ecotoxicity database. The summaries of environmental effects studies of 2,4-D are presented in **Appendix F**.

3. Exposure Assessment

3.1 Label Application Rates and Intervals

Crop-specific management practices for all of the assessed uses of 2,4-D were used for modeling, including application rates, number of applications per year, application intervals, and the first application date for each crop (**Table 3.1**). The date of first application was developed based on several sources of information including data provided by BEAD, a summary of individual applications from the CDPR PUR data, and Crop Profiles maintained by the USDA. More detail on the crop profiles may be found at http://www.ipmcenters.org/CropProfiles/. As depicted in **Figure 3.1**, most of the 2,4-D applications were made in the first quarter of the year from 1999 to 2006.

Table 3.1 2,4-D Uses Assessed for California, Modeling Scenario, Application Rates and Timing				
Master Label Use Category and Detailed Uses[1]	**2,4-D Forms for Which the Use is Labeled**	**PRZM/EXAMS Scenario (first app date)**	**Method[2]**	**Application Rate (interval between applications)**
Orchard Uses				
Nut Orchards, Pistachios	Acid, DMA, TIPA, IPA, DEA, Na	CA Almond wirrig STD (10-Feb)	G	2 apps @ 2 lb a.e./acre (30-day interval)
Filberts	Acid, DMA, TIPA, IPA, DEA, Na	CA Almond wirrig STD (10-Feb)	G	4 apps @ 0.5 lb a.e./acre[3] (30-day interval)
Grapes	Acid, DMA, TIPA, IPA, DEA, Na	CA Grapes STD (1-Mar)	G	1 app @ 1.36 lb a.e./acre
Grapes (wine grapes)	Acid, DMA, TIPA, IPA, DEA, Na	CA Wine Grapes RLF (1-Mar)	G	1 app @ 1.36 lb a.e./acre
Blueberries	Acid, DMA, TIPA, IPA, DEA, Na	CA Wine Grapes RLF (5-Mar)	G	1 post-emergence app @ 1.4 lb a.e./acre and 1 post-harvest app @ 1.4 lb a.e./acre
Stone and Pome Fruits	Acid, DMA, TIPA, IPA, DEA, Na	CA Fruit wirrig STD (1-Mar)	G	2 apps @ 2 lb a.e./acre (75-day interval)
Citrus	IPE	CA Citrus STD (1-Mar)	G	1 app @ 0.1 lb a.e./acre
			A	1 app @ 0.1 lb a.e./acre
Agricultural – Food Crop Uses				
Field Corn, Popcorn	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Corn OP (15-Mar)	G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)
			A	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)
Sweet Corn	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Corn OP (15-Mar)	G	1 app @ 1 lb a.e./acre March 15; 1 app @ 0.5 lb a.e./acre April 29
			A	1 app @ 1 lb a.e./acre on March 15; and 1 app @ 0.5 lb a.e./acre on April 29
Potatoes Fresh market only	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Potato RLF (1-Apr)	G	2 apps @ 0.07 lb a.e./acre (10-day interval)
			A	2 apps @ 0.07 lb a.e./acre (10-day interval)
Sugarcane[4]	Acid, DMA, TIPA, IPA, DEA, Na	CA Sugar beet wirrig OP (20-Jan)	G	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre (20-day interval)
			A	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre (20-day interval)
Cereal Grains Wheat, Barley, Millet, Oats, Rye	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Wheat RLF (10-Feb)	G	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)
			A	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)

Table 3.1 2,4-D Uses Assessed for California, Modeling Scenario, Application Rates and Timing				
Master Label Use Category and Detailed Uses[1]	**2,4-D Forms for Which the Use is Labeled**	**PRZM/EXAMS Scenario (first app date)**	**Method[2]**	**Application Rate (interval between applications)**
Grain or Forage Sorghum	Acid, DMA, TIPA, IPA, DEA, Na	CA Wheat RLF (10-Feb)	G	1 post-emergence app @ 1.0 lb a.e./acre
			A	1 post-emergence app @ 1.0 lb a.e./acre
	2-EHE, BEE	CA Wheat RLF (10-Feb)	G	1 post-emergence app @ 0.5 lb a.e./acre
			A	1 post-emergence app @ 0.5 lb a.e./acre
Hops	Acid, DMA, TIPA, IPA, DEA, Na	OR hops STD (10-Apr)	G	3 apps @ 0.5 lb a.e./acre (30-day interval)
			A	3 apps @ 0.5 lb a.e./acre (30-day interval)
Asparagus	Acid, DMA, TIPA, IPA, DEA, Na	CA Row Crop RLF (1-Apr)	G	2 apps @ 2 lb a.e./acre (30-day interval)
			A	2 apps @ 2 lb a.e./acre (30-day interval)
Fallowland and Crop Stubble	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Row Crop RLF (1-Aug)	G	2 apps @ 2 lb a.e./acre (30-day interval)
			A	2 apps @ 2 lb a.e./acre (30-day interval)
Agricultural – Non-food Crop Uses				
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Rangeland Hay RLF (1-Mar)	G	2 apps @ 2 lb a.e./acre (30-day interval)
Non-agricultural Uses				
Non-cropland Fencerows, Hedgerows, Roadsides, Ditches, Rights-of-way, Utility power lines, Railroads, Airports, Industrial sites, and Other non-crop areas	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Right-of-Way RLF (20-Feb)	G	1 app @ 4 lb a.e./acre
			A	1 app @ 4 lb a.e./acre
Forestry Forest site preparation, Forest roadsides, Brush control, Established conifer release including Christmas trees and reforestation areas	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Forestry RLF (1-Mar)	G	1 app @ 4 lb a.e./acre
			A	1 app @ 4 lb a.e./acre
Tree and Brush	Acid, DMA, TIPA,	CA Forestry RLF	G	1 app @ 4 lb a.e./acre

Table 3.1 2,4-D Uses Assessed for California, Modeling Scenario, Application Rates and Timing				
Master Label Use Category and Detailed Uses[1]	**2,4-D Forms for Which the Use is Labeled**	**PRZM/EXAMS Scenario (first app date)**	**Method**[2]	**Application Rate (interval between applications)**
Control Alder, Ash, Aspen, Birch, Blackgum, Cherry, Elm, Oak, Sweetgum, Tulip poplar, Willow, and Others	IPA, DEA, Na, 2-EHE, BEE	(1-Mar)	A	1 app @ 4 lb a.e./acre
Ornamental Turf Golf courses, Cemeteries, Parks, Sports fields, Turfgrass, Lawns and other grass areas	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Turf RLF (1-Mar)	G	2 apps @ 1.5 lb a.e./acre (21-day interval)
			A	2 apps @ 1.5 lb a.e./acre (21-day interval)
Grass Grown for Seed and Sod	Acid, DMA, TIPA, IPA, DEA, Na, 2-EHE, BEE	CA Turf RLF (1-Mar)	G	2 apps @ 2 lb a.e./acre (21-day interval)
			A	2 apps @ 2 lb a.e./acre (21-day interval)
Direct Application to Water Uses				
Rice	Acid, DMA, TIPA, IPA, DEA, Na	Direct water application (Rice model)[5]	G & A	1 app @ 1.5 lb a.e./acre
Aquatic Weed Control Surface application or subsurface injection for submersed weeds	Acid, DMA, TIPA, IPA, DEA, Na, BEE	Direct water application (Modeled using aerobic aquatic degradation rates)[5]	G & A	1 app @ 10.8 lb a.e./acre foot
Aquatic Weed Control Irrigation ditchbank application	Acid, DMA, TIPA, IPA, DEA, Na, BEE	Direct water application (Modeled using aerobic aquatic degradation rates)[5]	G & A	2 app @ 2.0 lb a.e./acre (30-day interval)
Aquatic Weed Control Surface application for floating and emergent aquatic weeds	Acid, DMA, TIPA, IPA, DEA, Na, BEE	Direct water application (Modeled using aerobic aquatic degradation rates)[5]	G & A	2 app @ 4.0 lb a.e./acre (21-day interval)

[1]Uses are derived from Master Label for Reregistration of 2,4-Dichlorophenoxyacetic Acid Uses – Supported by the 2,4-D Industry and IR-4.
[2]G = ground application. A = aerial application.
[3]The Master Label indicates a maximum single application rate of 1.0 lb a.e./100 gallons spray for filberts, SRRD verified that this rate is equivalent to a maximum single application rate of 0.5 lb a.e./acre, which represents a conservative estimate.
[4]Because EFED does not currently have a PRZM/EXAMS scenario for CA Sugarcane, sugarcane uses were modeled using the CA Sugar beet scenario as a surrogate.
[5]Details of the aquatic modeling and EEC estimation for direct application to water uses are discussed in **Sections 3.2.3** and **3.2.4.**

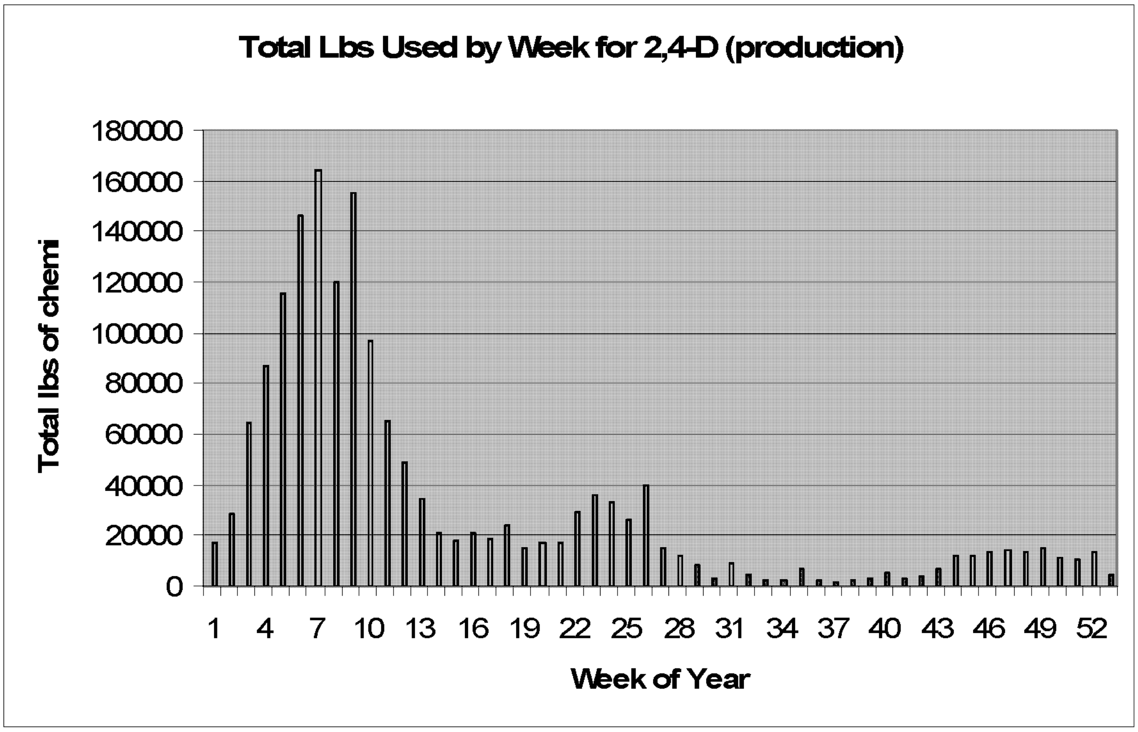


Figure 3.1 Total Pounds of 2,4-D Applied to Agricultural Production by Week from 1999 - 2006 based on CDPR PUR data. *Note: week 1 corresponds to January 1-7.*

3.2 Aquatic Exposure Assessment

3.2.1 Surface Water Modeling Approach and Inputs for 2,4-D Acid (all scenarios except rice and direct water application)

The appropriate PRZM and EXAMS input parameters for 2,4-D were selected from the environmental fate data submitted by the registrant and in accordance with U.S. EPA OPP EFED water model parameter selection guidelines (Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides, Version II, February 28, 2002). Input parameters can be grouped by physico-chemical properties and environmental fate data, application information, and scenarios. 2,4-D physical properties, environmental fate data, and other model parameters used as the inputs for PRZM and EXAMS are listed in **Table 3.2**.

Table 3.2 Summary of PRZM/EXAMS Modeling Inputs for 2,4-D Acid		
Fate Property	**Value**	**Source**
Molecular Weight	221 g/mol	Product Chemistry
Henry's constant	1.02×10^{-8} atm-m^3/mol	Product Chemistry
Vapor Pressure	1.4×10^{-7} torr	Product Chemistry
Solubility in Water	569 mg/L	Product Chemistry

Table 3.2 Summary of PRZM/EXAMS Modeling Inputs for 2,4-D Acid		
Fate Property	**Value**	**Source**
Photolysis in Water	13 days	MRID 41125306
Aerobic Soil Metabolism [1]	6.2 days	MRID 00116625 MRID 43167501
Hydrolysis	Stable	MRID 41007301
Aerobic Aquatic Metabolism (water column)	45 days	MRID 42025301 MRID 42979201 MRID 44188601
Anaerobic Aquatic Metabolism (benthic) [2]	231 days	MRID 43356001
K_{OC} [3]	61.7 mL/g	MRID 42045302 MRID 00112937 MRID 44117901
Chemical Application Method (CAM)	1 for ground applications 2 for foliar applications	EFED Guidance[4]
Application Efficiency	0.99 for ground applications 0.95 for aerial applications	EFED Guidance
Spray Drift Fraction	0.01 for ground applications 0.05 for aerial applications	EFED Guidance

[1]Upper 90th Percentile based on acceptable aerobic metabolism half lives of 1.44, 2.92, 4.5, 12.4, 4.38, 1.99, and 1.7 days.
[2]Single value to multiply by 3
[3]Average Koc value
[4]Inputs determined in accordance with EFED "*Guidance for Chemistry and Management Practice Input Parameters for Use in Modeling the Environmental Fate and Transport of Pesticides*" dated February 28, 2002

3.2.2 Surface Water Modeling Approach and Inputs for 2,4-D Ester Drift Only and Drift+Runoff (all scenarios except rice and direct water applications)

EFED's strategy for bridging the fate data requirements for the ester and amine salt forms of 2,4-D to the acid form was supported by laboratory data, which indicated rapid conversion of the amine salt and ester forms of 2,4-D to the acid form. The sodium salt form was considered to be equivalent to the acid form. However, it was noted at the time of the establishment of the fate strategy that 2,4-D esters may persist under acidic aquatic conditions. A condition of the establishment of the bridging strategy was that terrestrial field dissipation studies should be conducted using 2,4-D DMA and 2,4-D EHE. Review of the terrestrial field dissipation studies indicate that the study authors reported that 2,4-D DMA converts rapidly to 2,4-D acid (in many instances, conversion occurred in the tank mix), although it appears the analytical method may not have been able to detect 2,4-D DMA. The terrestrial field dissipation data for 2,4-D EHE indicate that the ester form may persist in the field for several days with half-lives ranging between 1 and 14 days and a median half-life of 2.9 days. In addition, the abiotic hydrolysis studies for the 2,4-D esters indicates that ester hydrolysis to 2,4-D acid is pH dependent with no hydrolysis occurring under acid or neutral conditions (as an example 2,4-D EHE hydrolyzes at pH 5 with a half-life of 99 days and the hydrolysis half-life at pH 7 is 48 days, while hydrolysis at pH 9 was 52 hours). However, hydrolysis soil slurry data indicate that dissipation in a non-sterile water body will occur at all pHs, and published literature indicates that 2,4-D esters in natural waters degrade rapidly with an average half-life of less than 3 hours. Registrant sponsored research indicates the 2,4-D esters (ethylhexyl, isopropyl, butylethyl) degrade rapidly ($t_{1/2}$< 24 hours) in soil slurries, aerobic aquatic environments, and anaerobic, acidic aquatic environments. Several field studies show phenoxy herbicide esters are more persistent under extremely dry soil [< soil wilting point (~15 bars)] conditions (Smith and Hayden, 1980; Smith, 1972; Smith,1976) while in moist soils [~50 to 80% field capacity (~0.3 bars)] and soil slurries, phenoxy herbicide esters degraded rapidly (>85% degradation) during a 48 hour incubation period. These degradation rates raise the concern of the impact of the drift of the esters of 2,4-D to aquatic environments when spray is applied to terrestrial systems. To address these concerns, two additional modeling approaches were utilized to account for potential ester exposures in the aquatic environments. For uses that allow for 2,4-D ester (BEE, EHE, or IPE) applications, a *drift only scenario* and a *drift+runoff scenario* were modeled. Chronic EECs were not provided in this scenario because both registrant and open literature data indicate that hydrolysis of the esters in a non-sterile water body will occur at all pHs in a relatively short time frame (< 48 hours).

Drift of 2,4-D esters to the standard aquatic pond was modeled for each scenario assuming 5% spray drift for aerial application and 1% spray drift for ground application (as per EFED guidance). The amount of loading for each scenario was estimated by converting the application rate (determined by reviewing ester labels only) to the drift loading and multiplying the application amount (2.24 kilograms per hectare for turf) by the drift (5% for aerial application). The resulting loading to the standard pond (0.112 kg to the 1 hectare pond as an example) was converted to an acute concentration by dividing the loading to the standard pond with a surface area of one hectare by the volume of the pond (20,000,000 liters). The resulting concentration represents the maximum instantaneous concentration predicted by direct drift from the application to the pond.

To account for the potential for runoff during the time in which 2,4-D EHE may remain in the field, EFED conducted additional modeling with PRZM/EXAMS to assess the potential for aquatic organisms to be exposed to 2,4-D EHE when applied to the same terrestrial crops as modeled in the ester drift scenario. Model inputs for 2,4-D EHE are listed in the **Table 3.3.** As with the drift only scenario, chronic EECs were not generated.

Table 3.3 PRZM/EXAMS Input Parameters for 2,4-D EHE			
Model Parameter	**Value**	**Comments**	**Source**
Aerobic Soil Metabolism $t_{1/2}$	24 days	estimated upper 90th percentile [1]	MRID 42059601
Aerobic Aquatic Degradation $t_{1/2}$ (KBACW)	48 days	2 x the aerobic soil metabolism degradation rate	Estimated per EFED Guidance [2]
Anaerobic Aquatic Degradation $t_{1/2}$ (KBACS)	Stable	No data	Estimated per EFED Guidance [2]
Aqueous Photolysis $t_{1/2}$	128 days		MRID 42749702
Hydrolysis $t_{1/2}$	48 days		MRID 42735401
Koc	10500 ml/g		Estimated by EpiSuite Software
Molecular Weight	333.26		Product Chemistry
Water Solubility	0.32 mg/L		Product Chemistry
Vapor Pressure	4.57×10^{-6} mm Hg		Product Chemistry
Henry's Law Constant	5.78×10^{-5} atm-m^3/mole		Product Chemistry

[1]Three times (Upper 90th Percentile) based on single soil half life estimated from acceptable laboratory volatility study of 8 days.
[2]From *A Guidance for Chemistry and Management Practice Input Parameters for Use in Modeling the Environmental Fate and Transport of Pesticides,* dated at February 28, 2002.

3.2.3 Surface Water Modeling Approach and Inputs for Rice Scenario

The use of 2,4-D on rice was modeled using a Tier I approach developed by EFED. A more complete discussion of the rice model may be found in the EFED policy memorandum dated May 8, 2007. The model involves an assumption of uniform application of pesticide to a rice paddy and calculates an EEC in the water column that could potentially be released from the paddy. EFED guidance recommends using this EEC for both acute and chronic exposures use on rice. For compounds that degrade rapidly into degradates that are not of risk concern, the chronic EEC is expected to be conservative. The formula of the Tier I Rice Model v1.0 is as follows:

$$C_w = \frac{m_{ai}'}{0.00105 + 0.00013 K_d}$$

and, if appropriate:

$$K_d = 0.01 K_{oc}$$

where:

C_w = water concentration [µg/L]
m_{ai}' = mass applied per unit area [kg/ha]
K_d = water-sediment partitioning coefficient [L/kg]
K_{oc} = organic carbon partitioning coefficient [L/kg]

2,4-D is registered for use in rice paddies for the acid and amine salt forms (esters are not registered for rice use) with a maximum seasonal application rate of 1.5 lb a.e./A. Modeling of this use rate results in an estimated 2,4-D concentration in the rice paddy of **1486 µg a.e./L**. This model was calibrated to be conservative for most pesticides at the edge of the paddy. The lack of consideration for degradation, dilution, and dispersion may affect estimated concentrations downstream from the rice paddies. However, the exact level of conservativeness has not been fully evaluated in the context of regionally-dependent management practices, pesticide management practices, and universe of pesticide fate properties. Once released from the paddy, the concentrations are expected to decrease due to degradation, dilution, and dispersion.

The EEC derived by modeling 2,4-D use on rice is higher than concentrations detected in the surface water monitoring data evaluated as part of this assessment. However, analytical results of pond water after the direct application of 2,4-D reported in an aquatic field dissipation study (MRID 43491601) on rice submitted by the registrant indicate that initial concentrations (equivalent to the instantaneous estimate above) were as high as 2343 µg a.e./L with a mean concentration reported as 1372 µg a.e./L, suggesting that the model estimates are comparable to measured concentrations.

3.2.4 Surface Water Modeling Approach and Inputs for Direct Application Scenario

Because there are no existing modeling scenarios for direct application to water, a first approximation of an EEC was predicted assuming direct application to the standard pond. For this assessment, EFED utilized a first-order decay model to estimate average concentrations, which incorporates degradation based on an acceptable aerobic aquatic metabolism study ($t_{1/2}$ = 15 days, used input value of 45 days per EFED Guidance) for the EFED standard pond with no flow. EFED assumed that 2,4-D is uniformly applied to the EFED standard pond with a surface area of 1 hectare and a volume of 20,000,000 liters. Peak concentrations were determined using the target concentration (if provided in the label) or by calculation based on application rate and pond volume assuming instantaneous mixing of chemical. The 21-day average and 60-day average concentrations were calculated assuming first-order dissipation from aerobic aquatic degradation. An equation representing first-order decay was used to estimate average concentrations:

$$concentration = \frac{C_0 \times \left(1 - e^{-kt}\right)}{kt}$$

where: Co = initial concentration,
k = first-order aerobic aquatic degradation rate (= 0.00064),
t = time (in hours).

The interpretation of the label for aquatic weed control (surface application or subsurface injection for submersed weeds) is that the target rate for 2,4-D use is based on concentration and not application rate. In order to account for this scenario, it was assumed that 2,4-D would be applied at a rate to meet the target concentration of 4000 µg/L. This assumption would be applicable across all water bodies since the target rate is based on a rate per acre foot of water (10.8 lb a.e./acre-foot) and would be independent of water body geometry/volume. This scenario included the assumption of uniform application across the entire water body. Modeling for this scenario predicts direct water application of 2,4-D will yield surface water concentrations of 2,4-D in the EFED standard pond of **4000 µg a.e./L** for peak, **3417 µg a.e./L** for the 21-day average, and **2610 µg a.e./L** for the 60-day average after a single application of 2,4-D. Although multiple applications are permitted, only a single application was modeled. Therefore, EECs would exceed those calculated in this assessment if multiple applications are made.

Other application scenarios in which water applications would occur are 'irrigation ditchbanks' and 'surface application for floating and emergent aquatic weeds.' Application rates for these uses are provided in lb a.e./acre, but were converted to a target (peak) concentration by assuming a uniform water depth of one foot. Twenty-one-day and 60-day average concentrations were calculated using the same formula as above (aquatic weed control, surface application, or subsurface injection). Again, for these uses multiple applications could be made in a calendar year; however, the EECs for only one application were calculated.

3.2.5 Surface Water Modeling Results and Estimated Aquatic EECs

The aquatic EECs for 2,4-D for the various scenarios and application practices are listed in **Table 3.4.** Two aquatic application scenarios with the highest peak water column concentrations are direct application to control aquatic weeds (range from 740 to 4000 µg a.e./L) and rice (1431 µg a.e./L). All other scenarios have peak concentrations less than 50 µg a.e./L, with the lowest value less than 0.5 µg a.e./L for citrus use.

Results of the drift loading of the 2,4-D esters to the standard aquatic pond are presented in **Table 3.5**. The 4 lb a.e./acre application rate predicts the highest peak concentrations of 11.2 µg a.e./L and 2.2 µg a.e./L, respectively for aerial applications and ground applications.

Results of the drift+runoff loading of the 2,4-D esters to the standard aquatic pond are presented in **Table 3.5**. The 4 lb a.e./acre application rate for forestry predicts the highest peak concentrations of 13.24 µg a.e./L and 7.14 µg a.e./L for aerial applications and ground applications, respectively.

Table 3.4 Aquatic EECs for 2,4-D Acid/Salt Uses in California						
Master Label Use Category[1]	PRZM/EXAMS Scenario (first app date)	Method[2]	Application Rate (interval between applications)	EEC (µg a.e./L)		
				Peak	21-day	60-day
Orchard Uses						
Nut Orchards, Pistachios	CA Almond wirrig STD (10-Feb)	G	2 apps @ 2 lb a.e./acre (30-day interval)	13.69	12.71	11.10
Filberts	CA Almond wirrig STD (10-Feb)	G	4 apps @ 0.5 lb a.e./acre[3] (30-day interval)	4.06	3.77	3.38
Grapes	CA Grapes STD (1-Mar)	G	1 app @ 1.36 lb a.e./acre	4.52	4.16	3.59
Grapes (wine grapes)	CA Wine Grapes RLF (1-Mar)	G	1 app @ 1.36 lb a.e./acre	2.94	2.70	2.34
Blueberries	CA Wine Grapes RLF (5-Mar)	G	1 post-emergence app @ 1.4 lb a.e./acre and 1 post-harvest app @ 1.4 lb a.e./acre	3.15	2.93	2.58
Stone and Pome Fruits	CA Fruit wirrig STD (1-Mar)	G	2 apps @ 2 lb a.e./acre (75-day interval)	6.98	6.35	5.30
Citrus[6]	CA citrus STD	A	1 app @ 0.1 lb a.e./acre	0.32	0.28	0.24
		G		0.08	0.08	0.07
Agricultural – Food Crop Uses						
Field Corn, Popcorn	CA Corn OP (15-Mar)	A	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)	12.17	11.13	9.84
		G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)	9.41	8.60	7.40
Sweet Corn	CA Corn OP (15-Mar)	A	1 app @ 1 lb a.e./acre March 15; 1 app @ 0.5 lb a.e./acre April 29	9.57	8.91	8.00
		G	1 app @ 1 lb a.e./acre on March 15; and 1 app @ 0.5 lb a.e./acre on April 29	7.41	6.90	6.00
Potatoes	CA Potato RLF (1-Apr)	A	2 apps @ 0.07 lb a.e./acre (10-day interval)	0.418	0.379	0.304
		G	2 apps @ 0.07 lb a.e./acre (10-day interval)	0.119	0.108	0.087
Sugarcane[4]	CA Sugar beet wirrig OP (20-Jan)	A	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre	33.31	31.28	27.40
		G	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre	25.85	24.25	21.02
Cereal Grains	CA Wheat RLF (10-Feb)	A	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	23.43	21.82	18.85
		G	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	21.39	19.89	17.19

Table 3.4 Aquatic EECs for 2,4-D Acid/Salt Uses in California						
Master Label Use Category[1]	**PRZM/EXAMS Scenario (first app date)**	**Method[2]**	**Application Rate (interval between applications)**	**EEC (µg a.e./L)**		
				Peak	**21-day**	**60-day**
Grain or Forage Sorghum	CA Wheat RLF (10-Feb)	A	1 post-emergence app @ 1.0 lb a.e./acre	18.61	17.33	14.96
		G	1 post-emergence app @ 1.0 lb a.e./acre	17.00	15.82	13.66
Hops	OR hops STD (10-Apr)	A	3 apps @ 0.5 lb a.e./acre (30-day interval)	4.62	4.19	3.69
		G	3 apps @ 0.5 lb a.e./acre (30-day interval)	1.73	1.55	1.33
Asparagus	CA Row Crop RLF (1-Apr)	A	2 apps @ 2 lb a.e./acre (30-day interval)	20.14	18.51	16.87
		G	2 apps @ 2 lb a.e./acre (30-day interval)	12.62	11.77	10.85
Fallow land and Crop Stubble	CA Row Crop RLF (1-Aug)	A	2 apps @ 2 lb a.e./acre (30-day interval)	10.70	9.70	8.32
		G	2 apps @ 2 lb a.e./acre (30-day interval)	2.16	1.95	1.69
Agricultural – Non-food Crop Uses						
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	CA Rangeland Hay RLF (1-Mar)	G	2 apps @ 2 lb a.e./acre (30-day interval)	13.02	12.10	10.52
Non-agricultural Uses						
Non-cropland	CA Right-of-Way RLF (20-Feb)	A	1 app @ 4 lb a.e./acre	46.66	43.76	38.62
		G	1 app @ 4 lb a.e./acre	39.02	36.54	32.24
Forestry, Tree and Brush Control	CA Forestry RLF (1-Mar)	A	1 app @ 4 lb a.e./acre	24.98	23.49	21.03
		G	1 app @ 4 lb a.e./acre	15.92	14.99	13.42
Ornamental Turf	CA Turf RLF (1-Mar)	A	2 apps @ 1.5 lb a.e./acre (21-day interval)	12.96	12.12	10.81
		G	2 apps @ 1.5 lb a.e./acre (21-day interval)	5.55	5.17	4.61
Grass Grown for Seed and Sod	CA Turf RLF (1-Mar)	A	2 apps @ 2 lb a.e./acre (21-day interval)	14.87	13.81	12.06
		G	2 apps @ 2 lb a.e./acre (21-day interval)	6.17	5.72	5.28
Direct Application to Water Uses						
Rice	Direct water application (Rice model)	G & A	1 app @ 1.5 lb a.e./acre	1486		
Aquatic Weed Control[5] Surface application or	Direct water application (Modeled using aerobic aquatic degradation rates)	G & A	1 app @ 10.8 lb a.e./acre foot	4000	3417	2610

Table 3.4 Aquatic EECs for 2,4-D Acid/Salt Uses in California						
Master Label Use Category[1]	**PRZM/EXAMS Scenario (first app date)**	**Method[2]**	**Application Rate (interval between applications)**	**EEC (µg a.e./L)**		
				Peak	**21-day**	**60-day**
subsurface injection for submersed weeds						
Aquatic Weed Control[5] Irrigation ditchbank application	Direct water application (Modeled using aerobic aquatic degradation rates)	G & A	2 app @ 2.0 lb a.e./acre (30-day interval)	740	632	483
Aquatic Weed Control[5] Surface application for floating and emergent aquatic weeds	Direct water application (Modeled using aerobic aquatic degradation rates)	G & A	2 app @ 4.0 lb a.e./acre (21-day interval)	1480	1264	966

[1]Uses are derived from Master Label for Reregistration of 2,4-Dichlorophenoxyacetic Acid Uses – Supported by the 2,4-D Industry and IR-4.
[2]G = ground application. A = aerial application.
[3]The Master Label indicates a maximum single application rate of 1.0 lb a.e./100 gallons spray for filberts, SRRD verified that this rate is equivalent to a maximum single application rate should of 0.5 lb a.e./acre, which represents a conservative estimate.
[4]Because EFED does not currently have a PRZM/EXAMS scenario for CA Sugarcane, sugarcane uses were modeled using the CA Sugar beet scenario as a surrogate.
[5] EECs from one application were calculated even though multiple applications are permitted.
[6] Although only IPE is labeled for citrus use, EFED is modeling exposure to acid as it is expected that most aquatic exposure will be to the acid, not 2,4-D IPE.

Table 3.5 Peak EECs for 2,4-D Esters in Surface Water Due to Drift Only and Drift+Runoff from All Applicable PE5 Modeling Scenarios					
Master Label Use Category[1]	**PRZM/EXAMS Scenario (first app date)**	**Method[2]**	**Application Rate (interval between applications)[3]**	**Peak EEC (µg a.e./L)**	
				Drift	**Drift+ Runoff**
Orchard Uses					
Citrus	CA citrus STD	A	1 app @ 0.1 lb a.e./acre	0.28	0.28
		G		0.055	0.055
Agricultural – Food Crop Uses					
Field Corn, Popcorn	CA Corn OP (15-Mar)	A	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)	4.2	4.66
		G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15 (pre-harvest)	0.83	2.70
Sweet Corn	CA Corn OP (15-Mar)	A	1 app @ 1 lb a.e./acre March 15; 1 app @ 0.5 lb a.e./acre April 29	2.8	3.11
		G	1 app @ 1 lb a.e./acre on March 15; and 1 app @ 0.5 lb a.e./acre on April 29	0.55	1.80
Potatoes	CA Potato RLF (1-Apr)	A	2 apps @ 0.07 lb a.e./acre (10-day interval)	0.196	0.19
		G	2 apps @ 0.07 lb a.e./acre (10-day interval)	0.0385	0.039
Cereal Grains	CA Wheat RLF (10-Feb)	A	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	3.5	5.09
		G	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	0.69	3.32
Grain or Forage Sorghum	CA Wheat RLF (10-Feb)	A	1 post-emergence app @ 0.5 lb a.e./acre	1.4	2.04
		G	1 post-emergence app @ 0.5 lb a.e./acre	0.28	1.33
Fallow land and Crop Stubble	CA Row Crop RLF (1-Aug)	A	2 apps @ 2 lb a.e./acre (30-day interval)	5.6	5.5
		G	2 apps @ 2 lb a.e./acre (30-day interval)	1.1	1.1
Agricultural – Non-food Crop Uses					
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	CA Rangeland Hay RLF (1-Mar)	G	2 apps @ 2 lb a.e./acre (30-day interval)	1.1	1.27
Non-agricultural Uses					
Non-cropland	CA Right-of-Way RLF (20-Feb)	A	1 app @ 4 lb a.e./acre	11.2	11.13
		G	1 app @ 4 lb a.e./acre	2.2	6.37

Table 3.5 Peak EECs for 2,4-D Esters in Surface Water Due to Drift Only and Drift+Runoff from All Applicable PE5 Modeling Scenarios					
Master Label Use Category[1]	PRZM/EXAMS Scenario (first app date)	Method[2]	Application Rate (interval between applications)[3]	Peak EEC (µg a.e./L)	
				Drift	Drift+ Runoff
Forestry, Tree and Brush Control	CA Forestry RLF (1-Mar)	A	1 app @ 4 lb a.e./acre	11.2	13.25
		G	1 app @ 4 lb a.e./acre	2.2	7.14
Ornamental Turf	CA Turf RLF (1-Mar)	A	2 apps @ 1.5 lb a.e./acre (21-day interval)	4.2	4.14
		G	2 apps @ 1.5 lb a.e./acre (21-day interval)	0.83	0.83
Grass Grown for Seed and Sod	CA Turf RLF (1-Mar)	A	2 apps @ 2 lb a.e./acre (21-day interval)	5.6	5.51
		G	2 apps @ 2 lb a.e./acre (21-day interval)	1.1	1.11

[1]Uses are derived from Master Label for Reregistration of 2,4-Dichlorophenoxyacetic Acid Uses – Supported by the 2,4-D Industry and IR-4.
[2]G = ground application. A = aerial application.
[3]Modeled EECs reflect exposure due to a single application of the chemical.

3.2.6 Groundwater Modeling of 2,4-D Acid

Based on SCIGROW modeling, the 2,4-D concentration in ground water is estimated to be **0.0311 µg a.e./L**. The result is based on inputs of 6.2 days for aerobic soil metabolism half-life, 13.23 for Koc, and a total annual application rate of 4 lb a.e./acre.

3.2.7 Existing Monitoring Data

A critical step in the process of characterizing Estimated Environmental Concentrations is comparing the modeled estimates with available surface water monitoring data. Most of this monitoring data is non-targeted (*i.e.*, study was not specifically designed to capture 2,4-D concentrations in high use areas). 2,4-D data from the USGS NAWQA program (http://water.usgs.gov.nawqa) and data from the California Department of Pesticide Regulation (CDPR) are included in this assessment. Typically, sampling frequencies employed in monitoring studies are insufficient to document peak exposure values. This, coupled with the fact that these data are not temporally or spatially correlated with pesticide application times and/or areas, limits the utility of these data in estimating exposure concentrations for risk assessment. These monitoring data are characterized in terms of general statistics including number of samples, frequency of detection, maximum concentration, and mean from all detections, where that level of detail is available.

3.2.7.1 USGS NAWQA Surface Water Data

Surface water monitoring data from the United States Geological Survey (USGS) NAWQA program was accessed in July 2008 and all data for the State of California were downloaded. A total of 264 water samples were analyzed for 2,4-D. Of these samples, 55

(20.8%) had positive detections of 2,4-D greater than or equal to 0.1 µg/L; among these detections, 3 were equal to or higher than 1 µg/L. The maximum 2,4-D detection was 1.39 µg/L in the Arcade Creek near Del Paso Heights in Sacramento County. The second highest concentration was 1.2 µg/L, which was detected in the salt slough at Highway 165 near Stevinson in Merced County. The third highest was 1 µg/L, which was detected in San Joaquin River near Patterson in Stanislaus County. Data are summarized by county in **Table 3.6.** In summary, there was no clear pattern in 2,4-D detections from different use sites because 2,4-D was detected in a number of different types of watersheds (agricultural, urban, mixed and other) as classified by the USGS land use information.

Table 3.6 Summary of 2,4-D Detections from NAWQA Sampling Data in California

County	Number of samples	Number < 0.10 µg/L	Highest Concentration (µg/L)
Alpine	4	4	
El Dorado	4	4	
Merced	68	64	1.2
Nevada	4	4	
Orange	6	0	0.27 (other 5 < 0.15)
Sacramento	62	42	1.39
San Bernardino	5	0	all reported < 0.15
San Joaquin	31	29	0.2
Stanislaus	54	45	1
Sutter	2	2	
Yolo	24	15	0.78

3.2.7.2 USGS NAWQA Groundwater Data

Groundwater monitoring data from the United States Geological Survey (USGS) NAWQA program were accessed in July 2008, and all data for the state of California was downloaded. A total of 210 water samples were analyzed for 2,4-D. Of these samples, 180 samples were identified as less than 0.035 µg/L; the other 30 were reported with measurements less than 0.15 µg/L.

3.2.7.3 California Department of Pesticide Regulation (CDPR) Data

Pesticide monitoring studies in surface water were primarily carried out by the California Department of Pesticide Regulation (CDPR), Environmental Hazard Assessment Program (EHAP), United States Geological Survey (USGS), and the Central Valley Regional Water Quality Control Board. Data from these and other studies are documented in EHAP's surface water database (SURF). Surface water monitoring data for 2,4-D was accessed and extracted from the CDPR on June 28, 2008. A total of 437 samples were available. Of these samples, 2,4-D was detected in 2 samples with greater than 2.0 µg/L (2.78 µg/L and 2.1 µg/L); both were located in Yolo County. The other two detections that were greater than 1.0 µg/L were 1.39 µg/L (Sacramento County) and 1.2 µg/L (Merced County). Approximately 5% of the samples (23) ranged between 0.1 – 0.78 µg/L. Approximately 90% of samples (394) reported the value of 0.

3.2.7.4 Atmospheric Monitoring Data

Based on 2,4-D's low vapor pressure (1.47×10^{-7} mm Hg @ 25 ^{0}C) and Henry's Law Constant (1.02×10^{-8} atm-m3/mol @ 25^{0}C), volatilization loss of 2,4-D from soil and water systems is expected to be insignificant. Based on relatively low volatility and high sensitivity to photolytic degradation, 2,4-D is not expected to continue long-range transport. Considering the uses of 2,4-D in California, all 2,4-D formulations are classified in terms of vapor pressure. Acid and salt forms are classed as "non-volatile" salts. Ester formulations are classified as either "high-volatile" or "low-volatile." All 2,4-D forms are classified as toxic air contaminants (TAC) according to CDPR (http://www.cdpr.ca.gov/docs/risk/priot.pdf). Two 2,4-D air monitoring studies were funded by California Department of Food and Agriculture in 1980. The first study showed that no positive 2,4-D air monitoring results were observed in a wide area of San Joaquin Valley (Simpson *et al.,* 1980). The second study also showed that no 2,4-D dimethylamine salt or isobutyl ester was detected in any of the samples collected (Neher *et al.,* 1980).

3.2.8 Downstream Dilution Analysis

As previously stated (**Section 2.7**), for 2,4-D, both the initial area of concern and the action area are considered to be the entire state of California. Due to the fact that the 2,4-D labels allow for aquatic uses in multiple types of water bodies, multiple applications within a specific watershed may occur within the same time frame. As a result, there is potentially no input of "2.4-D clean" water to dilute existing concentrations of 2,4-D downstream because it could be applied in the downstream waterbodies as well. Therefore, no credible watershed dilution can be done. For that reason, a downstream dilution analysis was not conducted.

3.3 Terrestrial Animal Exposure Assessment

T-REX (Version 1.4.1) is used to calculate dietary and dose-based EECs of 2,4-D for birds, mammals, and terrestrial invertebrates. T-REX simulates a 1-year time period. For this assessment, spray and granular applications of 2,4-D are considered.

Terrestrial EEC modeling inputs for foliar application formulations of 2,4-D were calculated by T-REX and summarized in **Tables 3.7.a and 3.7.b**. In addition to usage input values (application rates, number of applications, and application intervals), T-REX also utilizes a foliar dissipation half-life to estimate exposure. If chemical specific foliar dissipation data are not available, EFED uses a default half-life of 35 days (Willis and McDowell, 1987). Willis and McDowell (1987) did provide a foliar dissipation half-life for 2,4-D of 8.8 days. In addition, several forest field dissipation studies submitted to the Agency reported half-lives on foliage ranging from 33 to 42 days (MRIDs 439547-02, 439083-03 and 439271-01). Because study limitations created a great deal of uncertainty in these half-lives (*e.g.,* foliage only sampled from understory, some pre-treatment samples tested positive for 2,4-D, and concentrations were determined on a wet weight basis), EFED utilized the 8.8-day half-life for terrestrial organism exposure estimation.

EFED included the aquatic application scenarios (rice and aquatic weed control) in the terrestrial exposure assessment. Often the treated water bodies will be quite shallow, making them accessible to terrestrial organisms. It is also likely that some 2,4-D will be deposited off the target site and onto the land adjoining the treated water bodies.

For modeling purposes, exposures of the CRLF and AW to 2,4-D through contaminated food items are estimated using the EECs for the small bird (20 g), which consumes small insects. Dietary-based and dose-based exposures of potential prey of the CRLF and the AW are assessed using the small mammal (15 g), which consumes short grass. In addition, dietary-based and dose-based exposures of potential avian prey of the AW are assessed using the small birds (20 g), which consumes short grass. Upper-bound Kenega nomogram values reported by T-REX for these organism types are used for derivation of EECs for the CRLF and the AW and their potential prey (**Tables 3.7.a** and **3.7.b**). T-REX is also used to calculate EECs for terrestrial insects exposed to 2,4-D. Dietary-based EECs calculated by T-REX for small and large insects (units of a.e./g) are used to bound an estimate of exposure to insects (**Table 3.7.b**). A sample output from T-REX is available in **Appendix J**.

Exposure calculated as mg a.e./sq ft is provided for all granular applications (**Table 3.8**). For granular applications, exposure is only estimated for a single application.

Table 3.7.a Upper-bound Kenega Nomogram EECs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D

Modeling Scenario	Method[1]	Application Rate	EECs for CRLF and AW[2]		EECs for Mammalian Prey[3] (Indirect effects to CRLF and AW)	
			Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)	Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)
Orchard Uses						
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	295	336	525	501
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	75	85	132	126
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	184	209	326	311
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	207	236	368	351
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	271	308	481	459
Citrus	A/G	1 app @ 0.1 lb a.e./acre	14	15	24	23
Agricultural – Food Crop Uses						
Field Corn, Popcorn	A/G	1 app @ 1.0 lb a.e./acre March 15,	203	231	360	344

Table 3.7.a Upper-bound Kenega Nomogram EECs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D						
Modeling Scenario	Method[1]	Application Rate	EECs for CRLF and AW[2]		EECs for Mammalian Prey[3] (Indirect effects to CRLF and AW)	
			Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)	Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)
		1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15				
Sweet Corn	A/G	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	135	154	240	229
Potatoes	A/G	2 apps @ 0.07 lb a.e./acre (10-day interval)	14	16	24	23
Sugarcane	A/G	2 apps @ 2 lb a.e./acre (20-day interval)	326	371	579	552
Cereal Grains	A/G	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	169	192	300	286
Grain or Forage Sorghum	A/G	1 post-emergence app @ 1.0 lb a.e./acre	135	154	240	229
Hops	A/G	3 apps @ 0.5 lb a.e./acre (30-day interval)	74	85	132	126
Asparagus	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	295	336	525	501
Fallowland and Crop Stubble	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	295	336	525	501
Agricultural – Non-food Crop Uses						
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	295	336	525	501
Non-agricultural Uses						
Non-cropland	A/G	1 app @ 4 lb a.e./acre	540	615	960	915
Forestry	A/G	1 app @ 4 lb a.e./acre	540	615	960	915
Tree and Brush Control	A/G	1 app @ 4 lb a.e./acre	540	615	960	915
Ornamental Turf	A/G	2 apps @ 1.5 lb a.e./acre (21-day interval)	241	275	429	409
Grass Grown for Seed and	A/G	2 apps @ 2 lb a.e./acre (21-day interval)	322	366	572	545

Table 3.7.a Upper-bound Kenega Nomogram EECs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D						
Modeling Scenario	Method[1]	Application Rate	EECs for CRLF and AW[2]		EECs for Mammalian Prey[3] (Indirect effects to CRLF and AW)	
			Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)	Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)
Sod						
Direct Application to Water Uses						
Rice	A/G	1 app @ 1.5 lb a.e./acre	203	231	360	343
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[5]	7290	8303	12960	12356
Aquatic Weed Control	A/G	2 app @ 2 lb a.e./acre (30-day interval)	295	336	525	501
Aquatic Weed Control	A/G	2 app @ 4 lb a.e./acre (21-day interval)	643	733	1144	1090

[1]G = ground application. A = aerial application.
[2]EECs based on small bird (20 g) which consumes small insects.
[3]EECs based on small mammal (15 g) which consumes short grass.
[4]These EECs also apply for terrestrial invertebrates (small insects).
[5]Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre.

Table 3.7.b Upper-bound Kenega Nomogram EECs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D						
Modeling Scenario	Method[1]	Application Rate	EECs for Avian Prey (Indirect Effects to AW)[2]		EECs for Terrestrial Invertebrate Prey (Indirect Effects to CRLF and AW)	
			Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)	Small Insects (mg a.e./kg-insect)	Large Insects (mg a.e./kg-insect)
Orchard Uses						
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	525	598	295	33
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	132	151	75	8
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	326	371	184	20
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	368	419	207	23
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	481	548	271	30
Citrus	A/G	1 app @ 0.1 lb a.e./acre	24	27	14	2
Agricultural – Food Crop Uses						
Field Corn,	A/G	1 app @ 1.0 lb a.e./acre	360	410	203	23

Table 3.7.b Upper-bound Kenega Nomogram EECs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D						
Modeling Scenario	**Method[1]**	**Application Rate**	**EECs for Avian Prey (Indirect Effects to AW)[2]**		**EECs for Terrestrial Invertebrate Prey (Indirect Effects to CRLF and AW)**	
			Dietary-based EEC (mg a.e./kg-diet)	**Dose-based EEC (mg a.e./kg-bw)**	**Small Insects (mg a.e./kg-insect)**	**Large Insects (mg a.e./kg-insect)**
Popcorn		March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15				
Sweet Corn	A/G	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	240	273	135	15
Potatoes	A/G	2 app @ 0.07 lb a.e./acre (10-day interval)	24	28	14	2
Sugarcane	A/G	2 apps @ 2 lb a.e./acre (20-day interval)	579	660	326	36
Cereal Grains	A/G	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	300	342	169	19
Grain or Forage Sorghum	A/G	1 post-emergence app @ 1.0 lb a.e./acre	240	273	135	15
Hops	A/G	3 apps @ 0.5 lb a.e./acre (30-day interval)	132	151	75	8
Asparagus	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	525	598	295	33
Fallowland and Crop Stubble	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	525	598	295	33
Agricultural – Non-food Crop Uses						
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	525	598	295	33
Non-agricultural Uses						
Non-cropland	A/G	1 app @ 4 lb a.e./acre	960	1093	540	60
Forestry	A/G	1 app @ 4 lb a.e./acre	960	1093	540	60
Tree and Brush Control	A/G	1 app @ 4 lb a.e./acre	960	1093	540	60
Ornamental Turf	A/G	2 apps @ 1.5 lb a.e./acre (21-day interval)	429	488	241	27

Table 3.7.b Upper-bound Kenega Nomogram EECs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D						
Modeling Scenario	Method[1]	Application Rate	EECs for Avian Prey (Indirect Effects to AW)[2]		EECs for Terrestrial Invertebrate Prey (Indirect Effects to CRLF and AW)	
			Dietary-based EEC (mg a.e./kg-diet)	Dose-based EEC (mg a.e./kg-bw)	Small Insects (mg a.e./kg-insect)	Large Insects (mg a.e./kg-insect)
Grass Grown for Seed and Sod	A/G	2 apps @ 2 lb a.e./acre (21-day interval)	572	651	322	36
Direct Application to Water Uses						
Rice	A/G	1 app @ 1.5 lb a.e./acre	360	410	203	23
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[3]	12960	14760	7290	810
Aquatic Weed Control	A/G	2 app @ 2 lb a.e./acre (30-day interval)	525	598	295	33
Aquatic Weed Control	A/G	2 app @ 4 lb a.e./acre (30-day interval)	1144	1303	643	72

[1]G = ground application. A = aerial application.
[2]EECs based on small bird (20 g) which consumes short grass.
[3]Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre.

Table 3.8 EECs for Exposures of the CRLF and AW and their Prey to Granular Applications of 2,4-D (ground applications)		
Scenario	**Application Rate**	**EEC (mg a.e./ft^2)**
Agricultural Food Crop Uses		
Field Corn, Popcorn	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	15.62
Sweet Corn	1 app @ 1 lb a.e./acre March 15; 1 app @ 0.5 lb a.e./acre April 29	10.41
Grain or Forage Sorghum	1 post-emergence app @ 1.0 lb a.e./acre	10.41
Non-Agricultural Uses		
Non-cropland	1 app @ 4 lb a.e./acre	41.65
Ornamental Turf	2 apps @ 1.5 lb a.e./acre (21-day interval)	15.62
Grass Grown for Seed and Sod	2 apps @ 2 lb a.e./acre (21-day interval)	20.83
Direct Application to Water Uses		
Aquatic Weed Control	1 app @ 10.8 lb a.e./acre foot	562.30
Aquatic Weed Control	2 app @ 2 lb a.e./acre (30-day interval)	20.83
Aquatic Weed Control	2 app @ 4 lb a.e./acre (21-day interval)	41.65

3.4 Terrestrial Plant Exposure Assessment

TerrPlant (Version 1.2.2) is used to calculate EECs for non-target plant species inhabiting dry and semi-aquatic areas. Parameter values for application rate, drift assumption, and incorporation depth are based upon the use and related application method. A runoff value of 5% is utilized considering 2,4-D's solubility of 569 mg/L (>100 mg/L). For aerial and ground application methods, drift is assumed to be 5% and 1%, respectively. EECs relevant to terrestrial plants consider pesticide concentrations in drift and in runoff (**Table 3.9**).

Table 3.9 TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to 2,4-D via Runoff and Drift (single application only)						
Modeling Scenario	**Method[1]**	**Application Rate**	**Drift Value (%)**	**Dry Area EEC (lb a.e./acre)**	**Semi-aquatic Area EEC (lb a.e./acre)**	**Spray Drift EEC (lb a.e./acre)**
Orchard Uses						
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	1	0.12	1.02	0.02
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	1	0.03	0.25	0.005
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	1	0.082	0.014	0.694
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	1	0.084	0.714	0.014
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	1	0.12	0.02	1.02
Citrus	A/G	1 app @ 0.1 lb a.e./acre	1 5	0.006 0.01	0.051 0.055	0.001 0.005
Agricultural – Food Crop Uses						
Field Corn, Popcorn	A/G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	1 5	0.09 0.15	0.765 0.825	0.015 0.075
Sweet Corn	A/G	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	1 5	0.06 0.10	0.51 0.55	0.01 0.05
Potatoes	A/G	2 apps @ 0.07 lb a.e./acre (10-day interval)	1 5	0.004 0.007	0.036 0.039	0.0007 0.0035
Sugarcane	A/G	2 apps @ 2 lb a.e./acre (20-day interval)	1 5	0.12 0.20	1.02 1.10	0.02 0.01
Cereal Grains	A/G	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	1 5	0.075 0.125	0.638 0.688	0.013 0.063
Grain or Forage Sorghum	A/G	1 post-emergence app @ 1.0 lb a.e./acre	1 5	0.06 0.10	0.51 0.55	0.01 0.05
Hops	A/G	3 apps @ 0.5 lb a.e./acre (30-day interval)	1 5	0.03 0.03	0.25 0.25	0.005 0.05
Asparagus	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	1 5	0.12 0.20	1.02 1.10	0.02 0.01
Fallowland and Crop Stubble	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	1 5	0.12 0.20	1.02 1.10	0.02 0.01

Table 3.9 TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to 2,4-D via Runoff and Drift (single application only)						
Modeling Scenario	**Method[1]**	**Application Rate**	**Drift Value (%)**	**Dry Area EEC (lb a.e./acre)**	**Semi-aquatic Area EEC (lb a.e./acre)**	**Spray Drift EEC (lb a.e./acre)**
Agricultural – Non-food Crop Uses						
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	1	0.12	1.02	0.02
Non-agricultural Uses						
Non-cropland	A/G	1 app @ 4 lb a.e./acre	1 5	0.24 0.40	2.04 2.20	0.04 0.20
Forestry	A/G	1 app @ 4 lb a.e./acre	1 5	0.24 0.40	2.04 2.20	0.04 0.20
Tree and Brush Control	A/G	1 app @ 4 lb a.e./acre	1 5	0.24 0.40	2.04 2.20	0.04 0.20
Ornamental Turf	A/G	2 apps @ 1.5 lb a.e./acre (21-day interval)	1 5	0.09 0.15	0.765 0.825	0.015 0.075
Grass Grown for Seed and Sod	A/G	2 apps @ 2 lb a.e./acre (21-day interval)	1 5	0.12 0.20	1.02 1.10	0.02 0.01
Direct Application to Water Uses						
Rice	A/G	1 app @ 1.5 lb a.e./acre	1 5	0.09 0.15	0.765 0.825	0.015 0.075
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[3]	1 5	3.24 5.40	27.54 29.70	0.54 2.70
Aquatic Weed Control	A/G	1 app @ 2 lb a.e./acre	1 5	0.12 0.20	1.02 1.10	0.02 0.01
Aquatic Weed Control	A/G	1 app @ 4 lb a.e./acre	1 5	0.24 0.40	2.04 2.20	0.04 0.20

[1]G = ground application. A = aerial application.
[2]EECs calculated based on a single application. If crop labeled for multiple applications within a year, the highest single rate was used.
[3]Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre.

4. Effects Assessment

This assessment evaluates the potential for 2,4-D to directly or indirectly affect the CRLF and AW or modify their designated critical habitat. As previously discussed in **Section 2.7**, assessment endpoints for the effects determination for each assessed species include direct toxic effects on the survival, reproduction, and growth, as well as indirect effects, such as reduction of the prey base or modification of its habitat. In addition, potential modification of critical habitat is assessed by evaluating effects to the PCEs, which are components of the critical habitat areas that provide essential life cycle needs of each assessed species. Direct effects to the aquatic-phase CRLF are based on toxicity information for freshwater fish (or amphibian data if appropriate), while terrestrial-phase amphibian effects (terrestrial-phase CRLF) and reptiles (AW) are based on avian toxicity data, given that birds are generally used as a surrogate for terrestrial-phase amphibians and reptiles.

As described in the Agency's Overview Document (U.S. EPA, 2004), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include freshwater fish (used as a surrogate for aquatic-phase amphibians), freshwater invertebrates, aquatic plants, birds (used as a surrogate for terrestrial-phase amphibians and reptiles), mammals, terrestrial invertebrates, and terrestrial plants. Acute (short-term) and chronic (long-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on 2,4-D.

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (U.S. EPA, 2004). Open literature data presented in this assessment were obtained from the 2,4-D RED and the ECOTOX database, which was searched on June 30, 2008. In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

(1) the toxic effects are related to single chemical exposure;
(2) the toxic effects are on an aquatic or terrestrial plant or animal species;
(3) there is a biological effect on live, whole organisms;
(4) a concurrent environmental chemical concentration/dose or application rate is reported; and
(5) there is an explicit duration of exposure.

Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered. The degree to which open literature data are quantitatively or qualitatively characterized for the effects determination is dependent on whether the information is relevant to the assessment endpoints (*i.e.*, survival, reproduction, and growth) identified in **Section 2.8**. For example, endpoints such as behavior modifications are likely to be qualitatively evaluated, because quantitative relationships between modifications and reduction in species survival, reproduction, and/or growth are not available. Although the effects determination relies on endpoints that are relevant to the assessment endpoints of survival, growth, or reproduction, it is important to note that the full suite of sublethal endpoints potentially available in the effects literature (regardless of their significance to the assessment endpoints) are considered to define the action area for 2,4-D.

Citations of all open literature not considered as part of this assessment because they were either rejected by the ECOTOX screen or accepted by ECOTOX but not used (*e.g.*, the endpoint is less sensitive) are included in **Appendix G. Appendix G** also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this endangered species risk assessment.
A detailed spreadsheet of the available ECOTOX open literature data, including the full suite of lethal and sublethal endpoints is presented in **Appendix G**. Reviews of several of the ECOTOX and open literature studies are also included in **Appendix G**.

In addition to registrant-submitted and open literature toxicity information, other sources of information, including use of the acute probit dose response relationship to establish the probability of an individual effect and reviews of the Ecological Incident Information System (EIIS), are reviewed to further refine the characterization of potential ecological effects associated with exposure to 2,4-D. A summary of the available aquatic and terrestrial ecotoxicity information, use of the probit dose response relationship, and the incident information for 2,4-D are provided in **Section 4**.

Several degradates have been reported for 2,4-D but only a few have been identified and quantified. The Agency does not have concerns for any degradates of 2,4-D relative to human health issues as the tolerance expression is only expressed in terms of the 2,4-D parent based on the determination of the Metabolite Assessment Review Committee (MARC) Health Effects Division (HED) of OPP. There is no evidence that any 2,4-D degradates are of toxicological concern, and none of them (>10.0%) is found in a significant amount; therefore, this assessment is based on parent 2,4-D (acid, salts, and esters) only. Although ECOTOX data indicates the degradate 2,4-dichlorophenol (2,4-DCP) is more toxic than the parent for freshwater fish, freshwater invertebrates, and earthworms, the degradation of the parent only results in 3.5% available 2,4-DCP, which would not result in toxicity concerns for direct or indirect effects to the CRLF or AW.

2,4-D has registered products that contain multiple active ingredients. Analysis of the available open literature and acute oral mammalian LD_{50} data for multiple active ingredient products relative to the single active ingredient is provided in **Appendix A**. Based on a review of the available studies on 2,4-D mixtures in ECOTOX, it appears that the toxicity values presented in the mixture papers are no more sensitive than the toxicity of the single active ingredient, 2,4-D. The results of this analysis show that an assessment based on the toxicity of the single active ingredient of 2,4-D is appropriate.

A recent paper by Relyea (2008, see review in **Appendix G**) evaluated the effects of several pesticides alone and in combination on mesocosms containing aquatic communities consisting of zooplankton, phytoplankton, periphyton and larval amphibians (gray tree frogs, *Hyla versicolor* and leopard frogs, *Rana pipiens*). Each insecticide (malathion, carbaryl, chlorpyrifos, diazinon and endosulfan) and each herbicide (glyphosate, atrazine, acetochlor, metolachlor and 2,4-D acid) was evaluated singly as well as (a) all insecticides together, (b) all herbicides together, and (c) all insecticides and herbicides together. 2,4-D alone and the mixture of all herbicides did not appear to have any effects on the survival and metamorphosis of amphibian populations. However, there was a slight reduction in phytoplankton population (that effect was also seen in the acetochlor trial). The mixture of all insecticides and the mixture of all herbicides and insecticides caused a 99% reduction in leopard frogs and no reduction in gray tree frogs; because of this, gray tree frogs grew twice as large due to lack of competition.

4.1 Dioxin Contaminant Toxicity to Terrestrial Organisms

A key chemical intermediate in the manufacture of 2,4-D is 2,4-dichlorophenol (2,4-DCP), and the purity of this intermediate has a strong correlation to the purity of 2,4-D acid produced from it. In the manufacture of 2,4-DCP, multiple positions around the phenyl ring structure may be chlorinated. The desired positions for chlorination are carbons two and four of the phenyl ring, but the reaction may yield small quantities of compounds chlorinated at different positions. Certain combinations of these chlorinated structures may form precursors to the dioxin 2,3,7,8-TCDD.

According to 2,4-D registrants, since the 1990's, the manufacturing processes for 2,4-D and its chemical intermediate, dichlorophenol, have been modified, and those modifications decrease the chance that TCDD and PCDD are formed during the manufacturing process. Manufacture of the 2,4-DCP intermediate has been optimized by controlling processing conditions necessary to drive the chlorination reaction to the preferred two and four carbon positions, thereby limiting the formation of impurities that can lead to dioxin formation. Controlled temperature and residence time during the chlorination reaction, programmed addition of the chlorinating agent, and efficient agitation in the reaction vessel are processing factors that contribute to the purity of 2,4-DCP. Additionally, distillation of 2,4-DCP is a technique that may be employed post-chlorination to increase purity. Moreover, quality control sampling and analytical procedures are also utilized to verify product quality at various steps of the 2,4-DCP process. Results of testing of 2,4-DCP, performed in response to the Toxic Substances Control Act (TSCA) Dioxin/Furan Test Rule, showed no detectable concentrations of 2,3,7,8-substituted tetra- through hepta-CDD/CDFs.

In the manufacture of 2,4-D acid per se, there are additional process conditions and procedures that must be controlled to maximize yield and purity. Details regarding these measures are dependent on specific manufacturing methodologies and, as such, are protected under FIFRA Section 10 as Confidential Business Information.

The Agency's most recent evaluations of anticipated dioxin and furan residues resulting from 2,4-D applications are based on the concentrations of dioxins and furans present in technical grade 2,4-D as determined by review of analytical data submitted in response to the 1987 DCI. In those evaluations, completed in the early 1990's, the ratios of individual chlorodibenzo-p-dioxin (CDD; dioxin) or chlorodibenzo-p-furan (CDF; furan) contaminant concentrations to 2,4-D acid concentrations were calculated, and those ratios were used with 2,4-D tolerance expressions to calculate an anticipated residue in eggs, fruits, grains, kidney (hogs), meat (hogs), milk, nuts, poultry, and sugarcane for each detected dioxin or furan(SRRD RED, June 2005).

In addition to the above analysis for tolerance expressions, EFED completed a revised ecological risk assessment (May 31, 2005) to assess reproductive effects to piscivorous birds and mammals from exposure to PCDD and PCDF in technical 2,4-D and 2,4-D ester herbicides (see excerpts in **Appendix E**).

For each technical 2,4-D formulation for which the Agency received data, calculation of an anticipated dietary exposure was based on a worst-case scenario in which the highest anticipated residue was used, and an assumption was made that 100% of the diet consisted of the food item with the highest anticipated residue. Based on the confidential EFED risk assessment (May 31, 2005), there was no toxicity concerns for reproductive effects to piscivorous birds and mammals. The risk quotients were orders of magnitude below the LOC. The high runoff PRZM/EXAMS scenario (NC apple) was used to assess terrestrial runoff exposure. Because CA sites have lower runoff amounts than the NC sites, it is reasonable to assume that there will be no effect in the CRLF and AW assessment. Therefore, based on the calculation of dietary exposures using the worst-case scenarios, cancer, non-cancer, and reproductive (in birds and mammals) risks based on dietary exposure to dioxins and furans as contaminants of 2,4-D were considered to be of no toxicological concern.

4.2 Toxicity of 2,4-D to Aquatic Organisms

Table 4.1.a and **4.1.b** summarize the most sensitive aquatic toxicity endpoints, based on an evaluation of both the submitted studies and the open literature, as previously discussed. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the CRLF is presented below. Additional information is provided in **Attachment 3.** Because the AW is a terrestrial organism given its designated critical habitat as well as its prey base, the aquatic assessment does not include direct or indirect effects to the AW.

Toxicity to fish and aquatic invertebrates is categorized using the system shown in **Table 4.2** (U.S. EPA, 2004). Toxicity categories for aquatic plants have not been defined.

Table 4.1.a Freshwater Aquatic Toxicity Profile for 2,4-D Acid and Salts (DMA, TIPA, IPA, DEA, Na)				
Assessment Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**MRID (Author & Date)**	**Status/Comment**
Acute Direct Toxicity to Aquatic-phase CRLF	Common carp (*Cyprinus carpio)*	LC_{50} = 24.15 mg a.e./L	E006387 (Vardia and Durve, 1981)	Use quantitatively
Chronic Direct Toxicity to Aquatic-phase CRLF	Fathead Minnow (*Pimephales promelas*)	NOAEC = 14.2 mg a.e./L	417677-01 (Dill *et al.,* 1990)	Acceptable
Indirect Toxicity to Aquatic-phase CRLF via Acute Toxicity to Freshwater Invertebrates (*i.e.*, prey items)	Water Flea *(Daphnia magna)*	EC_{50} = 25 mg a.e./L	411583-01 (Alexander *et al.*, 1983)	Acceptable
Indirect Toxicity to Aquatic-phase CRLF via Chronic Toxicity to Freshwater Invertebrates (*i.e.,* prey items)	Water flea *(Daphnia magna)*	NOAEC = 16.05 mg a.e./L	420183-03 (Holmes *et al.*, 1991)	Acceptable
Indirect Toxicity to Aquatic-phase CRLF via Toxicity to Non-vascular Aquatic Plants	Freshwater Diatom *(Navicula pelliculosa)*	EC_{50} = 3.88 mg a.e./L	415059-03 (Hughes, 1990)	Acceptable
Indirect Toxicity to Aquatic-phase CRLF via Toxicity to Vascular Aquatic Plants	Water Milfoil *(Myriophyllum sibiricum)*	EC_{50} = 0.0131 mg a.e./L	E74985 (Roshon, 1997)	Use quantitatively

Table 4.1.b Freshwater Aquatic Toxicity Profile for 2,4-D Esters (2-EHE, BEE, IPE)				
Assessment Endpoint[a]	**Species**	**Toxicity Value Used in Risk Assessment**	**MRID (Author & Date)**	**Status/Comment**
Acute Direct Toxicity to Aquatic-Phase CRLF	Bluegill Sunfish (*Lepomis macrochirus)*	LC_{50} = 0.26 mg a.e./L	439307-01, 439103-01 (Drottar *et al.*, 1996)	Acceptable
Chronic Direct Toxicity to Aquatic-Phase CRLF	NA	NA	NA	NA
Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (*i.e.*, prey items)	Water Flea *(Daphnia magna)*	LC_{50} = 2.2 mg a.e./L	439306-01 (Drottar *et al.*, 1996)	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Chronic Toxicity to Freshwater Invertebrates (*i.e.,* prey items)	NA	NA	NA	NA

Table 4.1.b Freshwater Aquatic Toxicity Profile for 2,4-D Esters (2-EHE, BEE, IPE)				
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Non-vascular Aquatic Plants	Marine Diatom *(Skeletonema costatum)*	EC_{50} = 0.066 mg a.e./L	417352-04 (Hughes, 1990)	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Vascular Aquatic Plants	Duckweed *(Lemna gibba)*	EC_{50} = 0.33 mg a.e./L	417352-03 (Hughes, 1990)	Acceptable
[a]Although chronic aquatic toxicity data for esters were reviewed, most sensitive endpoints are not included in this toxicity profile because chronic risks of esters were not estimated because the hydrolysis soil slurry data indicate that dissipation in a non-sterile water body will occur at all PHs; therefore, long-term exposures are unlikely.				

Table 4.2 Categories of Acute Toxicity for Aquatic Animals	
LC_{50} (ppm)	**Toxicity Category**
< 0.1	Very highly toxic
> 0.1 - 1	Highly toxic
> 1 - 10	Moderately toxic
> 10 - 100	Slightly toxic
> 100	Practically non-toxic

4.2.1 Toxicity to Freshwater Fish and Aquatic-phase Amphibians

Although several registrant-submitted and ECOTOX studies evaluating the acute toxicity to aquatic-phase amphibians were reviewed, EFED determined that the use of freshwater fish data is preferable to the use of aquatic-phase amphibian data because it is unknown where the CRLF would fall on a species sensitivity distribution. Because amphibian data is not required from the registrant, it is EFED's standard approach to use freshwater fish as a surrogate for aquatic-phase amphibians. In addition, because acute amphibian data were less sensitive than acute freshwater fish data, the use of freshwater fish as a surrogate provides a more conservative estimation of risk to the aquatic-phase CRLF. Chronic aquatic-phase amphibian toxicity data were not available.

Freshwater fish toxicity data were also used to assess potential indirect effects of 2,4-D to the CRLF. Effects to freshwater fish resulting from exposure to 2,4-D have the potential to indirectly affect the CRLF via reduction in available food, as over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant, 1985).

4.2.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

Acute toxicity to freshwater fish can be summarized as practically non-toxic for the acid and salts and highly toxic for the esters. Definitive LC_{50} values for the acid and salts range from 101 to 2244 mg a.e./L; non-definitive LC_{50} values range from >81.6 to >830.0 mg a.e./L. The registrant-submitted study that reported the most sensitive toxicity value was for 2,4-D DEA salt with an LC_{50} of 101 mg a.e./L (MRID 0073-091-01); however, a more sensitive endpoint was found in the open literature.

The ester LC_{50} values range from 0.26 to 14.5 mg a.e./L. The most sensitive toxicity value was reported for two IPE studies (one with technical, and one with an end-use product), both with LC_{50} values of 0.26 mg a.e./L (MRID 439307-01, 439103-01). This value will be used to quantitatively estimate risks to the aquatic-phase CRLF.

4.2.1.2 Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

Chronic toxicity, based on larval survival and fish length from the early life stage studies, NOAECs range from 14.2 to 63.4 mg a.e./L for acids and salts. For risk estimation, the NOAEC of 14.2 mg a.e./L for DMA salt will be used (MRID 417677-01, most sensitive endpoint of length).

One full life cycle study was submitted to the Agency for 2,4-D EHE. This study resulted in a NOAEC of 0.0792 mg a.e./L, with the most sensitive endpoint of larval survival (MRID 417373-05). Although there was a registrant-submitted study that evaluated the chronic toxicity of esters to freshwater fish, this study will not be used in the assessment as chronic risks of esters were not evaluated due to the unlikelihood of long-term exposures (see Environmental Fate Strategy in **Section 2.4.1**).

4.2.1.3 Freshwater Fish: Open Literature Data

For acids and salts, acute LC_{50}/EC_{50} values ranged between 0.014 and 2884 mg a.e./L. However, some of these studies with low toxicity values were not scientifically sound or did not provide sufficient data to be used quantitatively in this risk assessment. A study that evaluated the effects of 2,4-D acid on the common carp resulted in a 96-hr LC_{50} of 24.15 mg a.e./L (E006387), which was more sensitive than the lowest reported value from registrant-submitted data; therefore, it will be used for quantitative acute risk estimation. One ECOTOX study (E000563) evaluated the chronic effects of 2,4-D potassium salt on several species of freshwater fish; however, insufficient data were available to determine a NOAEC.

For esters, acute LC_{50} values ranged from 0.302 to 8.8 mg a.e./L. None of these studies reported values that were more sensitive than the values reported in the registrant-submitted study. There were two studies that evaluated the chronic effects of esters (NOAECs ranged from 0.04 to 0.075 mg a.e./L); however, these studies will not be used in this risk assessment since chronic risks of esters were not evaluated due to the unlikelihood of long-term exposures (see Environmental Fate Strategy in **Section 1**).

4.2.1.4 Aquatic-phase Amphibian: Acute Studies

Two studies evaluating the effects of the acid and DMA on leopard frog tadpoles were submitted by the registrant and resulted in LC_{50}'s ranging from 278 to 359 mg a.e./L. For both BEE and EHE, registrant-submitted studies resulted in LC_{50} values of 0.505 mg a.e./L.

ECOTOX and open literature studies evaluating acute effects to aquatic-phase amphibians resulted in definitive endpoints (either LC_{50} or EC_{50}) ranging between 181 and 1962 mg a.e./L and a non-definitive endpoint of >38.9 mg a.e./L for acid and salts. No open literature studies that were conducted using an ester resulted in a lower toxicity than the registrant-submitted studies.

4.2.2 Toxicity to Freshwater Invertebrates

4.2.2.1 Freshwater Invertebrates: Acute Exposure Studies

Several registrant-submitted studies evaluating the acute effects of acid and salts on freshwater invertebrates provided an LC_{50} range of 25 to 642.8 mg a.e./L. For the purposes of risk estimation, the acid LC_{50} value of 25 mg a.e./L will be used (MRID 411583-01).

For esters, registrant-submitted studies reported a range of LC_{50} values from 2.2 to 11.88 mg a.e./L. For risk estimation, the IPE LC_{50} value of 2.2 mg a.e./L will be used (MRID 439306-01).

4.2.2.2 Freshwater Invertebrates: Chronic Exposure Studies

Two registrant-submitted studies were submitted resulting in a NOAEC range of 16.05 to 79 mg a.e./L for chronic effects of acid and salts. A third study did not provide a NOAEC; however, an LC_{50} = 75.7 mg a.e./L was determined. For the purposes of risk estimation, the DEA salt NOAEC of 16.05 mg a.e./L will be used (MRID 420183-03).

One chronic study was submitted by the registrant for esters (resulting in a NOAEC of 0.20 mg a.e./L for BEE; however, this study will not be use for chronic risk estimation as esters were not evaluated due to the unlikelihood of long-term exposures (see Environmental Fate Strategy in **Section 2.4.1**).

4.2.2.3 Freshwater Invertebrates: Open Literature Data

ECOTOX and open literature data for acute effects of acid and salts on freshwater invertebrates provided an LC_{50}/EC_{50} range of 0.1245 and 436.5 mg a.e./L. Although some toxicity values were more sensitive than registrant-submitted values, those studies were not scientifically sound or did not provide sufficient data to be used quantitatively in this risk assessment.

Definitive LC_{50}/EC50 values for esters ranged between 0.3036 and 4.4 mg a.e./L; one non-definitive LC_{50} value was > 69 mg a.e./L. None of these endpoints will be used for quantitative risk estimation. Although some toxicity values were more sensitive than registrant-submitted values, those studies were not scientifically sound or did not provide sufficient data to be used quantitatively in this risk assessment.

No chronic studies were reviewed in ECOTOX or open literature.

4.2.3 Toxicity to Aquatic Plants

Laboratory and field studies are the two types of studies used to evaluate the potential of 2,4-D to affect vascular and non-vascular aquatic plants. No field studies were available that evaluated the risks to aquatic plants at the time of this assessment. For non-vascular plant laboratory data, the toxicity values used for risk estimation can be observed from either freshwater or estuarine/marine species since guideline studies do not sufficiently explore the relative sensitivity of algae with regards to freshwater or estuarine/marine environment.

4.2.3.1 Aquatic Plants: Laboratory Data

Vascular Plants

Registrant-submitted Tier II studies reported effects of acid and salts to vascular plants provided an EC_{50} range of 0.2992 to 1.28 mg a.e./L. However, a more sensitive toxicity value was reported in open literature study; none of the registrant-submitted toxicity values will be used for estimation of risks of acids and salts to aquatic plants.

Two registrant-submitted Tier II studies reported an EC_{50} range of 0.33 to 0.3974 mg a.e./L for toxicity of esters to vascular plants. The 2-EHE EC_{50} of 0.33 mg a.e./L will be used for the purposes of risk estimation of esters to vascular plants (MRID 417352-03).

ECOTOX and open literature data for effects of acids and salts on aquatic vascular plants provided an EC_{50} range of 0.0131 and 0.334 mg a.e./L. Because the study providing the EC_{50} = 0.0131 mg a.e./L toxicity value was more sensitive than the value reported in the registrant-submitted study, this value will be used to quantitatively estimate the risks to aquatic vascular plants (E74985).

No studies were found in ECOTOX or open literature that evaluated the effects of esters on aquatic vascular plants.

Non-vascular Plants

For non-vascular aquatic plants, registrant-submitted Tier II studies reported effects of acid and salts with an EC_{50} range of 3.88 to 156.5 mg a.e./L. For risk estimation, the DMA salt EC_{50} of 3.88 mg a.e./L will be used (MRID 415059-03).

For ester toxicity to non-vascular aquatic plants, registrant-submitted Tier II studies reported an EC_{50} range from 0.066 to 19.8 mg a.e./L. The 2-EHE EC_{50} of 0.066 mg a.e./L will be used for the purposes of risk estimation (MRID 417352-04).

In addition to the Tier II studies, several Tier I studies were submitted and reviewed for the acid and IPE. Since the Tier II studies provided more detailed information about the toxicity of 2,4-D and most provided more sensitive toxicity values, they will be used in the risk assessment.

For effects of acids and salts on aquatic non-vascular plants, there were two studies in ECOTOX, but neither of these studies provided a definitive toxicity value that was less than the value reported in the registrant-submitted study. No studies were found in ECOTOX or open literature that evaluated the effects of esters on aquatic non-vascular plants.

4.3 Toxicity of 2,4-D to Terrestrial Organisms

Table 4.3 summarizes the most sensitive terrestrial toxicity endpoints, based on an evaluation of both the submitted studies and the open literature. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment is presented below. All registrant submitted studies are summarized in **Appendix F.**

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 4.4** (U.S. EPA, 2004). Toxicity categories for terrestrial plants have not been defined.

Table 4.3 Terrestrial Toxicity Profile for 2,4-D

Endpoint	Acute/ Chronic	Species	Toxicity Value Used in Risk Assessment	MRID (Author & Date)	Comment
Birds (surrogate for terrestrial-phase amphibians and reptiles)	A(gavage)	Bobwhite quail	LD_{50} = 298 mg a.e./kg-bw	442757-01 (Beavers, 1985)	Moderately toxic Acceptable, conducted using IPA
	A(dietary)	Bobwhite quail Mallard duck	LC_{50} > 3035 mg a.e./kg-diet	416444-02 (Driscoll *et al.*, 1990) 416444-03 (Driscoll *et al.*, 1990)	Slightly toxic Acceptable, both conducted using TIPA
	C	Bobwhite quail	NOAEC =962 LOAEC >962	415861-01 (Culotta, 1989)	No significant effects. Acceptable, conducted using acid
Mammals	A	Laboratory rat	LD_{50} = 441	414135-01	Moderately toxic, Acceptable, conducted using TIPA
	C	Laboratory rat	NOAEL = 5mg a.e./kg-bw/day	00150557 00163996	Decreased female body wt gain(F1) and male renal tubule alteration (F0 and F1); decreased pup weights

Table 4.3 Terrestrial Toxicity Profile for 2,4-D					
Endpoint	**Acute/ Chronic**	**Species**	**Toxicity Value Used in Risk Assessment**	**MRID (Author & Date)**	**Comment**
					Acceptable, conducted using acid
Terrestrial invertebrates	A	Honey bee, contact	LD_{50} >66 µg a.e./bee	445173-01	Acceptable, conducted using EHE
Terrestrial plants	N/A	Seedling Emergence Monocots	EC_{25} = 0.097 lb a.e./acre	471060-01 (Porch *et al.*, 2006)	Onion, dry weight Acceptable, conducted using DMA TEP
	N/A	Seedling Emergence Dicots	EC_{25}= 0.012 lb a.e./acre	471060-03 (Porch *et al.*, 2006)	Tomato, dry weight Acceptable, conducted using EHE TEP
	N/A	Vegetative Vigor Monocots	EC_{25} = 0.088 lb a.e./acre	471060-04 (Porch *et al.*, 2006)	Onion, dry weight Acceptable, conducted using EHE TEP
	N/A	Vegetative Vigor Dicots	EC_{25} = 0.0021 lb a.e./acre	471060-04 (Porch *et al.*, 2006)	Lettuce, dry weight Acceptable, conducted using EHE TEP
N/A: not applicable					

Table 4.4 Categories of Acute Toxicity for Terrestrial Organisms		
Categories of Acute Toxicity for Birds and Mammals		
Toxicity Category	**Oral LD_{50}**	**Dietary LC_{50}**
Very highly toxic	< 10 mg/kg	< 50 ppm
Highly toxic	10 – 50 mg/kg	50 - 500 ppm
Moderately toxic	51 – 500 mg/kg	501 - 1000 ppm
Slightly toxic	501 - 2000 mg/kg	1001 - 5000 ppm
Practically non-toxic	> 2000 mg/kg	> 5000 ppm
Categories of Acute Toxicity for Non-Target Insects		
Toxicity Category	**LC_{50}**	
Highly toxic	< 2 µg/bee	
Moderately toxic	2-11 µg/bee	
Practically nontoxic	>11 µg/bee	

4.3.1 Toxicity to Birds

As specified in the Overview Document, the Agency uses birds as a surrogate for reptiles and terrestrial-phase amphibians when toxicity data for each specific taxon are not available (U.S. EPA, 2004). No reptile or terrestrial-phase amphibian data were available for 2,4-D.

4.3.1.1 Birds: Acute Exposure (Mortality) Studies

Seven bobwhite quail gavage toxicity studies for various forms of 2,4-D were submitted to the Agency. Five had definitive LD_{50}s, ranging from 298 to 1578 mg a.e./kg-bw, and two had non-definitive LD_{50}s, which were >219 and >1380 mg a.e./kg-bw. Five mallard duck gavage toxicity studies were submitted to the Agency. All resulted in non-definitive LD_{50}s ranging from >314 to >5620 mg a.e./kg-bw. The LD_{50} of 298 mg a.e./kg-bw will be used for risk estimation (MRID 442757-01, conducted with IPA).

Nine bobwhite quail dietary toxicity studies for various forms of 2,4-D were submitted to the Agency. All had non-definitive LC_{50}s, ranging from >3035 to >8300 mg a.e./kg-diet. Eight mallard duck dietary toxicity studies were submitted to the Agency. All resulted in non-definitive LC_{50}s ranging from >3035 to >5620 mg a.e./kg-diet. The lowest LC_{50}s, both obtained from studies conducted with TIPA, will be used for risk estimation (MRIDs 416444-02 and 416444-03, no mortalities or overt signs of toxicity at any test concentration).

4.3.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

One reproductive study (bobwhite quail) was submitted to the Agency using 2,4-D acid. This study resulted in a NOAEC of 926 mg a.e./kg-diet, the highest concentration tested. No significant effects were noted at any concentration. This will be used for chronic risk estimation for all forms of 2,4-D.

4.3.1.3 Birds: Open Literature Data

None of the ECOTOX or open literature data provided acute toxicity information that was more sensitive than those values reported in the registrant-submitted data.

Several avian reproductive studies were available in ECOTOX. Although some toxicity values were more sensitive than registrant-submitted values, those studies were not scientifically sound or did not provide sufficient data to be used quantitatively in this risk assessment.

4.3.2 Toxicity to Mammals

4.3.2.1 Mammals: Acute Exposure (Mortality) Studies

Eight laboratory rat gavage toxicity studies for various forms of 2,4-D were submitted to the Agency. Six studies had definitive LD_{50}s ranging from 441 to 749 mg a.e./kg-bw; one study had a non-definitive LD_{50} > 579 mg a.e./kg-bw. One study was not included in the summary as it was conducted with an end-use product that contained a mixture of two active ingredients (2,4-D EHE and 2-ethylhexyl ester of 2-(2,4-dichlorophenoxy)propionic acid).

4.3.2.2 Mammals: Chronic Exposure (Growth, Reproduction) Studies

One two-generation reproductive study (laboratory rat) was submitted to the Agency using 2,4-D acid. This study resulted in a NOAEC of 5 mg a.e./kg-bw based on the parental effects of decreased female body weight gain (F1) and male renal tubule alteration (F0 and F1)and the offspring effects of decreased pup body weight. Reproductive effects had a NOAEC of 20 mg a.e./kg-bw based on increased gestation time.

4.3.2.3 Mammals: Open Literature Data

None of the ECOTOX or open literature data provided acute or chronic toxicity information that was more sensitive than those values reported in the registrant-submitted data.

Several mammalian reproductive studies were available in ECOTOX. Although some toxicity values were more sensitive than registrant-submitted values, most were not scientifically sound or did not provide sufficient data to be used quantitatively in this risk assessment.

In a study conducted using male Swiss mice (E93505), the NOAEC for 2,4-D acid was established at 1.7 mg a.e./kg-bw due to increases in chromosome aberrations in bone marrow and spermatocyte cells at higher doses (administered via oral gavage for up to five consecutive days). 2,4-D acid induced a dose-dependent increase in the percentage of sperm-head abnormalities; a NOAEC was established at 3.3 mg a.e./kg-bw (doses administered via oral gavage for five consecutive days). This study was not utilized for risk estimation as frank reproductive effects were not evaluated. However, it does indicate that genotoxic effects may occur at doses lower than the NOAEC established in the 2-generation reproduction study submitted by the registrant.

Also in this study (E93505), genotoxic effects were evaluated for the degradate, 2,4-DCP. Results indicated that the genotoxic effect of 2,4-DCP was weaker than that of 2,4-D. Statistically-significant increases in chromosome aberrations in bone marrow and in spermatocyte cells as well as increases in sperm-head abnormalities were observed following a single ip injection of 2,4-DCP at 180 mg/kg-bw. No other statistically-significant differences from the controls were indicated at the lower treatment levels.

4.3.3 Toxicity to Terrestrial Invertebrates

4.3.3.1 Terrestrial Invertebrates: Acute Contact Studies

Two honey bee acute contact studies were submitted to the Agency. For DMA and EHE, the studies resulted in LD_{50}s >83 and >66 µg a.e./bee, respectively. Percent mortality ranged from 1 to 3% in the DMA study, and from 1 to 35% in the EHE study.

4.3.3.2 Terrestrial Invertebrates: Open Literature Data

A study (E39264; Wahl and Ulm, 1983) found in ECOTOX was conducted in Germany in the early 1970's evaluating the effects of the feed quality of young bees and their ability to resist toxic effects of twenty formulations of various pesticides. The purpose of the study was to determine if a rich supply of higher protein feed fed to young bees would cause them to be less sensitive (higher LD_{50}'s) than bees fed a lower quality (lower protein) diet. Although this study had several variables and was meant to explore causes of observed bee mortality rather than to establish the acute toxicity value of a chemical, it does provide useful information about bees' toxic response when exposed to 2,4-D residues via consumption of contaminated food sources.

The authors studied the effects of several types of pollen fed in varying quantities (high quality food sources), as well as dried skim milk and sugar feed (low quality food sources). Because the OPPTS 850.3020 guideline study requires bees to be fed a diet of sugar water, the LD_{50}'s from this food type are relevant. The authors observed LD_{50}'s of 34.3 µg a.e./bee and 39.5 µg a.e./bee when fed sugar water and a 2,4-D Na end-use product. This study will be used to qualitatively characterize risk as EFED does not currently have methods to estimate bee exposure through ingestion.

Toxicity of 2,4-D acid and the degradate 2,4-DCP to mature earthworms (*Eisenia foetida)* was evaluated by Roberts and Dorough (E040531; 1984). 2,4-DCP was found to be more toxic to earthworms than the parent 2,4-D acid with LC_{50}'s of 4.4 (95% CI: 3.2-5.9) µg/cm^2 and 61.6 (95% CI: 41.0-92.4) µg/cm^2, respectively, in a 48-hr study. This study will be used to qualitatively characterize risk.

4.3.4 Toxicity to Terrestrial Plants

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature were reviewed for this assessment. No open literature data presented more sensitive results than those submitted by the registrants. Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated at both seedling emergence and vegetative life stages. Guideline studies generally evaluate toxicity to ten crop species. A drawback to these tests is that they are conducted on herbaceous crop species only, and extrapolation of effects to other species, such as the woody shrubs and trees and wild herbaceous species, contributes uncertainty to risk conclusions.

Commercial crop species have been selectively bred and may be more or less resistant to particular stressors than wild herbs and forbs. The direction of this uncertainty for specific plants and stressors, including 2,4-D, is largely unknown. Homogenous test plant seed lots also lack the genetic variation that occurs in natural populations, so the range of effects seen from tests is likely to be smaller than would be expected from wild populations.

The results of the Tier II seedling emergence and vegetative vigor toxicity tests on non-target plants are summarized in **Appendix F. Tables F16** to **F19** contain summary data for the most sensitive monocot and dicot endpoint for all seedling emergence and vegetative vigor studies conducted using technicals of various forms of 2,4-D. Tables containing results of all species tested using the technicals are provided in the EFED chapter of the 2,4-D RED.

Since the RED's completion, terrestrial plant studies were submitted to the Agency for end-use products of 2,4-D DMA and 2,4-D EHE. A summary of results for all species are contained in **Tables F20** to **F23**. The most sensitive species and endpoints from these studies will be used for risk estimation. As the studies were conducted with an end-use product, the exposure to the plants in the study will be similar to exposure to plants in the environment. Current study guidelines recommend using an end-use product for this reason. Adjuvants, surfactants, and other inactive ingredients have the potential to increase the toxicity of the active ingredient, relative to the effect of the active ingredient alone.

There did not appear to be any systematic differences in toxicity between the evaluated acid, salts, and esters for either the tests conducted using the technical or the tests conducted using the end-use products. Therefore, data from end-use product DMA studies and the end-use product EHE studies will be bridged and the most sensitive species and endpoints will be used for risk estimation.

In the RED assessment, risk estimation was conducted separately for the acid/salts and for the esters as differences in solubilities led to different inputs in the TerrPlant estimation program. Because there was no evidence of difference in toxicity since the salts dissociate to the acid very quickly (*e.g.,* 2,4-D amine salt dissociates in < 3 minutes) and the esters hydrolyze to the acid reasonably quickly (*e.g.,* 2,4-D esters in normal agriculture soil and natural water are short lived compounds, < 2.9 days), EFED will bridge the toxicity and the exposure estimation for terrestrial plants in this assessment.

4.4 Incident Database Review

A review of the EIIS database for ecological incidents involving 2,4-D acid, salts, and esters (PC Codes 030001, 030004, 030016, 030019, 030025, 030035, 030053, 030063 and 030066) was completed on December 16, 2008. A complete list of the incidents involving 2,4-D (acid, salts, and esters), including associated uncertainties, is included as **Appendix H**.

4.4.1 Terrestrial Incidents

Seven incidents for 2,4-D acid were reported for mammal and bird mortalities, which included multiple species, from 1991 – 2007 for uses on corn, agricultural areas, right-of-ways, turf/residential, home/lawn, and sunflowers (**Table 4.5**). Two of these incidents were the results of accidental misuse, and one report did not file a specific use. Mortality incidents were reported from exposure through drift and runoff, and one incapacitation incidence was reported from exposure through ingestion.

Based on three incident reports 2,4-D has been implicated as being toxic to mammals with possible and probable certainty for registered and undetermined use legalities.
In one incident report 2,4-D has been implicated as being toxic to birds with probable certainty for an undetermined use legality.

Table 4.5 Summary of 2,4-D Terrestrial Incidents in EIIS Database

Use Site/ Location/Year	Incident ID	Legality	Certainty	Species	Magnitude	Response	Exposure
Agricultural Area (Washington, UT) 1992	I000309-001	Undetermined	Possible	Chipmunk	Numerous	Mortality	Ingestion
				Dog	6	Mortality	Ingestion
				Horse	1	Incapacitation	Ingestion
				Horse	6	Mortality	Ingestion
				Squirrel	Numerous	Mortality	Ingestion
Agricultural Area (IL) 1970	B000150-002	Registered use	Probable	Fox Squirrel	2	Mortality	Runoff
Corn (Des Moines, IA) 1996	I004495-001	Misuse (accidental)	Highly Probable	Unknown bird	Unknown	Mortality	Drift
N/R (Durham, NC) 1992	I000008-001	Misuse (accidental)	Unlikely	Bluebird	3 nests full	Mortality	N/R
Sunflower (Lincoln, CO) 2006	I017576-001	Registered use	Unlikely	American kestrel	1	Mortality	Ingestion
				American robin	1	Mortality	Ingestion
				Common grackle	5	Mortality	Ingestion
				Horned lark	597	Mortality	Ingestion

Table 4.5 Summary of 2,4-D Terrestrial Incidents in EIIS Database							
Use Site/ Location/Year	**Incident ID**	**Legality**	**Certainty**	**Species**	**Magnitude**	**Response**	**Exposure**
				Kangaroo rat	Unknown	Mortality	Ingestion
				Lark bunting	Few	Mortality	Ingestion
				Mourning dove	1633	Mortality	Ingestion
				Red-winged blackbird	5	Mortality	Ingestion
				Sparrow	12	Mortality	Ingestion
				Unknown bird	150	Mortality	Ingestion
				Western meadow-lark	5	Mortality	Ingestion
Turf, residential (Lancaster , Pa) 2007	I019025-039	Undetermined	Possible	Rabbit	4	Mortality	Ingestion
Home/Lawn (Alamance, NC) 1991	I000799-003	Undetermined	Probable	Blackbird	Unknown	Mortality	Ingestion
				Bream	Hundreds	Mortality	Runoff
				Cardinal	Unknown	Mortality	Ingestion
				Duck	Hundreds	Mortality	Ingestion
				Turkey	Unknown	Mortality	Ingestion

4.4.2 Plant Incidents

For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. For 2,4-D, 140 of the 358 incidents reported were registered uses and 143 were of unknown legality. The majority of the reports were of possible to highly probable certainty. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. The majority of the reports were filed from 1990 – 2007. Only ten reports were filed prior to 1990.

For the 2,4-D acid, 269 incident reports were filed for a wide variety of terrestrial plants, particularly for uses on home/lawn, residential turf, corn, agricultural areas and right-of-ways. Other incidents included uses on barley, bean, cotton, orchard, ornamentals, pasture, pinto bean, potato, rangeland, rice, soybean, switch grass, tree farm, trees, wheat, yard, driveway, fence row, fields, grass, hay, hillside, municipal operations, and municipal sites. The reports were filed from 1949 – 2006 (only 10 reports were filed prior to 1990) with 66 misuses, 103 registered uses, and 100 uses of unknown legality. Plant damage, browning, and mortality were the main issues with drift and direct treatment as the main exposure routes.

For the 2,4-D DMA salt, 73 incident reports were filed for a wide variety of terrestrial plants, particularly for uses on home/lawn. Other incidents included uses on utility right-of-ways, rail right-of-ways, conservation reserve, rhododendron, yard and agricultural areas. The reports were filed from 1992 – 2002 with 7 accidental misuses, 25 registered uses, and 41 uses of unknown legality. Plant damage and mortality were the main issues with drift and direct treatment as the main exposure routes. Browning occurred for only one incident.

Fifteen incidents occurred for all other salts reported for 2,4-D which included 13 incidents for TIPA salt, 1 incident for DEA salt, and 1 incident for IPA salt . The above incidents were filed from 1993 -2007 with 2 misuses, 11 registered uses, and 2 uses of unknown legality. Incidents for TIPA salt were filed on a variety of terrestrial plants for uses on agricultural area, brome, corn fields, hay, pasture, peanut, rangeland, and soybean. Plant damage and mortality were the main issues with drift, direct treatment, persistence on crops, and carryover as exposure routes. Incidents for DEA salt and IPA salt were filed for uses on agricultural area and milo, respectively. No exposure route was reported for milo use; however, the agricultural area incident was due to drift exposure.

Only one incident was reported for 2-EHE ester, which was filed in 1998 as a registered use. Plant damaged occurred with drift as the main exposure route.

4.4.3 Aquatic Incidents

Twenty-six incidents were filed for 2,4-D acid, and 3 incidents were filed for 2,4-D DMA salt. The reports were filed from 1970 – 1997 with 5 misuses, 9 registered uses, 3 spills, and 12 uses of unknown legality. Out of 26 incidents reported for aquatic organisms for 2,4-D acid and DMA salt, six registered uses were reported with certainties of highly probable(2), probable(2) and possible (2). Incidences for 2,4-D were filed on aquatic organisms from runoff or drift.

All incidents resulted in mortality of aquatic organisms exposed to 2,4-D from runoff or drift. Incidents for 2,4-D were filed on aquatic organisms, which included the following species: Greengill, largemouth bass, bass, silver minnow, smallmouth bass, sunfish, catfish, crappie, perch, bream croaker, spot tail bass, carp, gizzard shad, salmon, American eel, blacknose dace, notropis minnow, minnow, white sucker, bluegill, mullet, drum, garfish, perch, crab, and watersnake. Use sites for the above aquatic organisms were reported on home/lawn, corn, agricultural areas, right-of-ways/railroad, lake, pond, spills, stream, sugar cane, tobacco, turf/golf course, athletic fields. Nine use sites were not reported.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations. Risk characterization is used to determine the potential for direct and/or indirect effects to the CRLF and AW or for modification of their designated critical habitats from the use of 2,4-D in CA. The risk characterization provides an estimation (**Section 5.1**) and a description (**Section 5.2**) of the likelihood of adverse effects; articulates risk assessment assumptions, limitations, and uncertainties; and synthesizes an overall conclusion regarding the likelihood of adverse effects to the assessed species or their designated critical habitats (*i.e.*, "no effect," "may affect and likely to adversely affect," or "may affect but not likely to adversely affect").

5.1 Risk Estimation

Risk is estimated by calculating the ratio of exposure to toxicity. This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (**Appendix I**). For acute exposures to the aquatic animals, as well as terrestrial invertebrates, the LOC is 0.05. For acute exposures to the birds (and, thus, reptiles and terrestrial-phase amphibians) and mammals, the LOC is 0.1. The LOC for chronic exposures to animals and acute exposures to plants is 1.0.

Acute and chronic risks to aquatic organisms are estimated by calculating the ratio of exposure to toxicity using 1-in-10 year EECs based on the label-recommended 2,4-D usage scenarios summarized in **Tables 3.4** and **3.5** and the appropriate aquatic toxicity endpoint from **Tables 4.1.a** and **4.1.b**. Acute and chronic risks to terrestrial animals and plants are estimated based on exposures resulting from applications of 2,4-D (**Tables 3.7.a, 3.7.b, 3.8** and **3.9**) and the appropriate toxicity endpoint from **Table 4.3**.

5.1.1 Exposures in the Aquatic Habitat

5.1.1.1 Direct Effects to Aquatic-phase CRLF

Because the AW is a terrestrial organism given its designated critical habitat as well as its prey base, the aquatic assessment does not include direct or indirect effects to the AW.

Direct effects to the aquatic-phase CRLF are based on peak EECs in the standard pond and the lowest acute toxicity value for freshwater fish. Separate RQs were calculated for the acid/salts (runoff+drift) and the esters (drift only and drift+runoff). In addition, risks to the aquatic-phase CRLF were estimated for direct applications to water. In order to assess direct chronic risks to the CRLF, 60-day EECs and the lowest acid/salts chronic toxicity value for freshwater fish were used. Due to the improbability of long-term exposures (see Environmental Fate Strategy in **Section 2.4.1**), chronic risks of esters were not estimated.

Acute and chronic RQ values did not exceed the acute LOC (0.05) and the chronic LOC (1.0) in any of the acid/salts modeled scenarios or drift only ester modeled scenarios. (**Appendix M**). For drift+runoff ester uses, only one scenario had an RQ that met the acute LOC; aerial Forestry and Tree and Brush Control uses (modeled by the CA Forestry RLF scenario) produced an RQ of 0.05 at the rate of 1 application at 4 lb a.e./acre (**Table 5.1.a**). For direct applications to water, the rice and aquatic weed control acid/salt and ester use RQs exceeded the acute LOC with RQs ranging from 0.06 to 15.38 (**Table 5.1.b**). **Table 5.1.c** summarizes the individual effect probabilities for the aquatic-phase CRLF, to represent 2,4-D acid, salt, and ester uses. Based on the LOC exceedances for scenarios listed in **Tables 5.1.a** through **5.1.c**, there is potential for 2,4-D uses to directly affect the aquatic phase of the CRLF.

Table 5.1.a Acute RQs for freshwater fish based on EECs for drift+runoff used to represent 2,4-D ester uses[1]

Master Label Use Category (EHE, BEE)	**PRZM/EXAMS Scenario (first app date)**	**Method[2]**	**Application Rate[3]**	**Peak EEC (µg/L)**	**Acute RQ**
Non-agricultural Uses					
Forestry, Tree and Brush Control	CA Forestry RLF (1-Mar)	G	1 app @ 4 lb a.e./acre	7.1353	0.03
		A	1 app @ 4 lb a.e./acre	13.249	**0.05***

*LOC exceedances (acute RQ ≥ 0.05) are bolded. Acute RQ = use-specific peak EEC / 0.26 mg a.e./L (MRID 439307-01, 439103-01). The most sensitive 2,4-D ester toxicity values were bridged for all use scenarios to calculate RQs.
[1]Chronic EECs are not modeled in this scenario because the hydrolysis soil slurry data indicate that dissipation in a non-sterile water body will occur at all PHs and therefore long-term exposures are unlikely.
[2]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.
[3]Esters are not persistent; only one application modeled due to rapid hydrolysis of EHE to the acid form.

Table 5.1.b Acute and chronic RQs for freshwater fish based on EECs for direct application to water to represent 2,4-D acid, salt, and ester uses

Master Label Use Category	**Model Scenario**	**Method[2]**	**Application Rate**	**Peak EEC (µg/L)**	**60-day EEC (µg/L)**	**Acute RQ**	**Chronic RQ**
Rice (acid and salts)	Direct water applications	G & A	1 app @ 1.5 lb a.e./ acre	1486[2]	N/A	**0.06***	0.11
Aquatic Weed Control (surface application or subsurface injection) (acid and salts)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[3]	2610	**0.17***	0.18
Aquatic Weed Control (surface application or subsurface injection) (esters only)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[3]	2610	**15.38***	NA[5]
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @2 lb a.e./acre (30-day interval)	740	483	**2.9***	NA[5]

Table 5.1.b Acute and chronic RQs for freshwater fish based on EECs for direct application to water to represent 2,4-D acid, salt, and ester uses							
Master Label Use Category	**Model Scenario**	**Method[2]**	**Application Rate**	**Peak EEC (µg/L)**	**60-day EEC (µg/L)**	**Acute RQ**	**Chronic RQ**
Aquatic Weed Control (acid and salts)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	966	**0.06***	0.07
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	966	**5.7***	NA[4]

*,+LOC exceedances (acute RQ ≥ 0.05; chronic RQ ≥ 1.0) are bolded. Acute RQ (acid and salts) = use-specific peak EEC / 24.15 mg a.e./L (E006387). Chronic RQ (acid and salts) = use-specific 60-day EEC / 14.2 mg a.e./L (MRID 417677-01). Acute RQ (esters) = use-specific peak EEC / 0.26 mg a.e./L (MRID 439307-01, 439103-01).
[1]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.
[2]Rice Model - the maximum water surface concentration is used to determine both acute and chronic toxicity.
[3]Aquatic weed control-peak water concentration: 4000 µg/L , 21-day average water concentration: 3417 µg/L, and 60-day average water concentration: 2610 µg/L. For ester direct application scenarios, 2,4-D acid input parameters were used to determine EEC. All other runoff and drift application scenarios used 2,4-D ester input parameters to determine EEC.
[4]Chronic EECs are not modeled in this scenario because the hydrolysis soil slurry data indicate that dissipation in a non-sterile water body will occur at all pHs and therefore long-term exposures are unlikely.

Table 5.1.c Summary of Direct Effect RQs for the Aquatic-phase CRLF[1], Individual Effect Probabilities to represent 2,4-D acid, salt, and ester uses				
Scenario	**Method[2]**	**Application Rate**	**Direct Effects Acute RQ***	**Probability of Individual Effect at RQ[3,4] (Confidence Interval)**
Non-agricultural Uses				
Forestry, Tree and Brush Control (esters only)	G/A	1 app @ 4 lb a.e./acre	**0.05***	1 in 8.29E+42 (6.05, 15.18)
Direct application to water Uses				
Rice (acid and salts)	G & A	1 app @ 1.5 lb a.e./ acre	**0.06***	1 in 1.02E+38 (6.05, 15.18)
Aquatic Weed Control (surface application or subsurface injection) (acid and salts)	G/A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	**0.17***	1 in 3.74E+03 (2, 9)
Aquatic Weed Control (surface application or subsurface injection) (esters only)	G/A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	**15.38***	1 in 1.00E+00 (6.05, 15.18)
Aquatic Weed Control (esters only)	G/A	2 app @2 lb a.e./acre (30-day interval)	**2.9***	1 in 1.00E+00 (6.05, 15.18)

Table 5.1.c Summary of Direct Effect RQs for the Aquatic-phase CRLF[1], Individual Effect Probabilities to represent 2,4-D acid, salt, and ester uses				
Scenario	**Method[2]**	**Application Rate**	**Direct Effects Acute RQ***	**Probability of Individual Effect at RQ[3,4] (Confidence Interval)**
Aquatic Weed Control (acid and salts)	G/A	2 app @ 4 lb a.e./ acre (21-day interval)	**0.06***	1 in 1.02E+38 (6.05, 15.18)
Aquatic Weed Control (esters only)	G/A	2 app @ 4 lb a.e./ acre (21-day interval)	**5.7***	1 in 1.00E+00 (6.05, 15.18)

*LOC exceedances (acute RQ $\geq$ 0.05) are bolded. Acute RQ (acid and salts) = use-specific peak EEC / 24.15 mg a.e./L (E006387). Acute RQ (esters) = use-specific peak EEC / 0.26 mg a.e./L (MRID 439307-01, 439103-01).
[1]RQs associated with acute and chronic direct toxicity to the CRLF are also used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items
[2]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.
[3]A probit slope and 95% confidence interval for the acute bluegill toxicity test for ester was available; therefore, the effect probability was calculated based on slope of 10.61 with a 95% confidence interval of (6.05, 15.18).
[4]A probit slope for the common carp toxicity test (acid and salts) was not available; therefore, the effect probability was calculated based on the default slope of 4.5 with a 95% confidence interval of (2, 9).

5.1.1.2 Indirect Effects to Aquatic-Phase CRLF via Reduction in Prey (Non-vascular Aquatic Plants, Aquatic Invertebrates, Fish, and Frogs)

5.1.1.2.1 Non-vascular Aquatic Plants

Indirect effects of 2,4-D to the aquatic-phase CRLF (tadpoles) via reduction in non-vascular aquatic plants in its diet are based on peak EECs from the standard pond and the lowest toxicity value (EC_{50}) for aquatic non-vascular plants.

There were no LOC exceedances except for direct applications to water uses. All RQs are provided in **Appendix M**, RQs resulting in LOC exceedances are provide in **Table 5.2**. These direct applications to water have the potential to indirectly affect the aquatic-phase CRLF through a reduction in food sources.

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Table 5.2 RQs for non-vascular plants based on EECs for direct application to water to represent 2,4-D acid, salt, and ester uses					
Master Label Use Category	**Model Scenario**	**Method[1]**	**Application Rate**	**Peak EEC (µg/L)**	**RQ**
Aquatic Weed Control (surface application or subsurface injection) (acid and salts)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[2]	**1.03***
Aquatic Weed Control (surface application or subsurface injection) (esters only)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[2]	**60.61***
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @ 2 lb a.e./acre (30-day interval)	740	**11.21***
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	**22.42***
*LOC exceedances (RQ ≥ 1.0) are bolded. RQ (acid and salts) = use-specific peak EEC / 3.88 mg a.e./L (MRID 415059-03). RQ (esters) = use-specific peak EEC / 0.066 mg a.e./L (MRID 417352-04). [1]G = ground application. A = aerial application. All applications are liquid unless otherwise specified. [2]Aquatic weed control-peak water concentration: 4000 µg/L , 21-day average water concentration: 3417 µg/L, and 60-day average water concentration: 2610 µg/L. For ester direct application scenarios, 2,4-D acid input parameters were used to determine EEC. All other runoff and drift application scenarios used 2,4-D ester input parameters to determine EEC.					

5.1.1.2.2 Aquatic Invertebrates

Indirect acute effects to the aquatic-phase CRLF via effects to prey (invertebrates) in aquatic habitats are based on peak EECs in the standard pond and the lowest acute toxicity value for freshwater invertebrates. Separate RQs were calculated for the acid/salts (runoff+drift) and the esters (drift+runoff and drift only). In addition, indirect risks to the aquatic-phase CRLF were estimated for direct applications to water. For chronic risks, 21-day EECs and the lowest chronic toxicity value for invertebrates were used to derive RQs for acid/salt uses. Due to the improbability of long-term exposures (see Environmental Fate Strategy in **Section 2.4.1**), chronic risks of exposure to esters were not estimated.

There were no acute or chronic LOC exceedances for acid/salt uses, drift+runoff ester uses, and drift only ester uses (**Appendix M).** For direct applications to water, rice and the aquatic weed control acid/salt and ester use RQs exceeded the acute LOC (**Table 5.3.a**).

Based on the results of probit analysis in **Table 5.3.b,** there is a significant chance (> 10%) that direct applications to water (aquatic weed control ester uses) will impact prey of the CRLF via direct effects on aquatic invertebrates as dietary food items. Based on the LOC exceedances and the results of the probit analysis, there is potential for 2,4-D uses to indirectly affect the aquatic phase of the CRLF through a reduction in invertebrate prey.

Table 5.3.a Acute and chronic RQs for freshwater invertebrates based on EECs for direct application to water to represent 2,4-D acid, salt, and ester uses

Master Label Use Category	Model Scenario	Method[1]	Application Rate	Peak EEC (µg/L)	21-day EEC (µg/L)	Acute RQ	Chronic RQ
Rice (acid and salts)	Direct water applications	G & A	1 app @ 1.5 lb a.e./acre	1486[2]	N/A	**0.06***	0.09
Aquatic Weed Control (surface application or subsurface injection) (acid and salts)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[3]	3417	**0.16***	0.21
Aquatic Weed Control (surface application or subsurface injection) (esters only)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[3]	3417	**1.82***	NA[4]
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @2 lb a.e./acre (30-day interval)	740	632	**0.34***	NA[4]
Aquatic Weed Control (acid and salts)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	1264	**0.05***	0.07
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	1264	**0.67***	NA[4]

*,+LOC exceedances (acute RQ ≥ 0.05; chronic RQ ≥ 1.0) are bolded. Acute RQ (acid and salts) = use-specific peak EEC / 25 mg a.e./L (MRID 411583-01). Chronic RQ (acid and salts) = use-specific 21-day EEC / 16.05 mg a.e./L (MRID 420183-03). Acute RQ (esters) = use-specific peak EEC / 2.2 mg a.e./L (MRID 439306-01).
[1]G = ground application. A = aerial application All applications are liquid unless otherwise specified.
[2]Rice Model - the maximum water surface concentration is used to determine both acute and chronic toxicity.
[3]Aquatic weed control-peak water concentration: 4000 µg/L , 21-day average water concentration: 3417 µg/L, and 60-day average water concentration: 2610 µg/L. For ester direct application scenarios, 2,4-D acid input parameters were used to determine EEC. All other runoff and drift application scenarios used 2,4-D ester input parameters to determine EEC.
[4]Chronic EECs are not modeled in this scenario because the hydrolysis soil slurry data indicate that dissipation in a non-sterile water body will occur at all PHs and therefore long-term exposures are unlikely.

Table 5.3.b Summary of Acute RQs Used to Estimate Indirect Effects to the CRLF via Direct Effects on Aquatic Invertebrates as Dietary Food Items (prey of CRLF juveniles and adults in aquatic habitats), Percent Effect Probabilities					
Master Label Use Category	**Model Scenario**	**Method[1]**	**Application Rate**	**Acute RQ**	**% Effect at RQ[2]**
Rice (acid and salts)	Direct water applications	G & A	1 app @ 1.5 lb a.e./acre	**0.06***	2%
Aquatic Weed Control (surface application or subsurface injection) (acid and salts)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	**0.16***	9%
Aquatic Weed Control (surface application or subsurface injection) (esters only)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	**1.82***	88%
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @2 lb a.e./acre (30-day interval)	**0.34***	2%
Aquatic Weed Control (acid and salts)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	**0.05***	1%
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	**0.67***	22%

*LOC exceedances (acute RQ ≥ 0.05; Acute RQ (acid and salts) = use-specific peak EEC / 25 mg a.e./L (MRID 411583-01). Acute RQ (esters) = use-specific peak EEC / 2.2 mg a.e./L (MRID 439306-01).
[1]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.
[2] For acid/salts, a probit slope and 95% confidence interval for the daphnia acute toxicity test was available; therefore, the effect probability was calculated based on the slope of 1.69 with a 95% confidence interval of (1.05, 2.34). For esters, a probit slope and 95% confidence interval for the daphnia acute toxicity test was not available; therefore, the effect probability was calculated based on the default slope of 4.5 with a 95% confidence interval of (2, 9).

5.1.1.2.3 Fish and Frogs

Fish and frogs also represent potential prey items of adult aquatic-phase CRLFs. RQs associated with acute and chronic direct toxicity to the CRLF (**Table 5.1.a** and **5.1.b**) are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Acute RQ values exceed the acute LOC in a few modeled scenarios for fish and frogs. Based on the LOC exceedances and the individual effects analysis listed in **Tables 5.1.a** through **5.1.c**, there is potential for 2,4-D uses to indirectly affect the aquatic-phase CRLF through a reduction in vertebrate prey (fish and frogs).

5.1.1.3 Indirect Effects to CRLF via Reduction in Habitat and/or Primary Productivity (Freshwater Aquatic Plants)

Indirect effects to the CRLF via direct toxicity to aquatic plants are estimated using the most sensitive non-vascular and vascular plant toxicity endpoints. Because there are no obligate relationships between the CRLF and any aquatic plant species, the most sensitive EC_{50} values, rather than NOAEC values, were used to derive RQs.

There were several LOC exceedances for acid/salt uses for vascular aquatic plants (**Table 5.4.a**). The RQs with LOC exceedances ranged from 1.05 to 3.56. There were no LOC exceedances for drift+runoff ester uses and drift only ester uses (**Appendix M**). For direct applications to water, the rice use and the acid/salt and ester aquatic weed control RQs exceeded the LOC with RQs ranging from 2.2 to 305.34 (**Table 5.4.b**). Based on the LOC exceedances, there is potential for 2,4-D uses to indirectly affect the aquatic-phase CRLF through a reduction in habitat and/or primary productivity.

Table 5.4.a RQs for vascular plants based on EECs for runoff and drift used to represent 2,4-D acid and salt uses

Master Label Use Category	PRZM/EXAMS Scenario (first app date)	Method[1]	Application Rate (interval between applications)	Peak EEC (µg/L)	RQ
Orchard Uses					
Nut Orchards, Pistachios	CA Almond wirrig STD (10-Feb)	G	2 apps @ 2 lb a.e./acre (30-day interval)	13.69	**1.05***
Agricultural – Food Crop Uses					
Sugarcane	CA Sugar beet wirrig OP (20-Jan)	G	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre	25.85	**1.97***
		A	1 pre-emergence and 1 post-emergence app @ 2 lb a.e./acre	33.31	**2.54***
Cereal Grains	CA Wheat RLF (10-Feb)	G	1 post-emergence app @ 1.25 lb a.e./acre; 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	21.39	**1.63***
		A	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	23.43	**1.79***
Grain or Forage Sorghum	CA Wheat RLF (10-Feb)	G	1 post-emergence app @ 1.0 lb a.e./acre	17.00	**1.30***
		A	1 post-emergence app @ 1.0 lb a.e./acre	18.61	**1.42***
Asparagus	CA Row Crop RLF (1-Apr)	G	2 apps @ 2 lb a.e./acre (30-day interval)	12.62	0.96

Table 5.4.a RQs for vascular plants based on EECs for runoff and drift used to represent 2,4-D acid and salt uses					
Master Label Use Category	**PRZM/EXAMS Scenario (first app date)**	**Method[1]**	**Application Rate (interval between applications)**	**Peak EEC (µg/L)**	**RQ**
		A	2 apps @ 2 lb a.e./acre (30-day interval)	20.14	**1.54***
Non-agricultural Uses					
Non-cropland	CA Right-of-Way RLF (20-Feb)	G	1 app @ 4 lb a.e./acre	39.02	**2.98***
		A	1 app @ 4 lb a.e./acre	46.66	**3.56***
Forestry, Tree and Brush Control	CA Forestry RLF (1-Mar)	G	1 app @ 4 lb a.e./acre	15.92	**1.22***
		A	1 app @ 4 lb a.e./acre	24.98	**1.91***
Grass Grown for Seed and Sod	CA Turf RLF (1-Mar)	G	2 apps @ 2 lb a.e./acre (21-day interval)	6.17	0.47
		A	2 apps @ 2 lb a.e./acre (21-day interval)	14.87	**1.14***

*LOC exceedances (RQ ≥ 1.0) are bolded. RQ = use-specific peak EEC / 0.0131 mg a.e./L (E74985). The most sensitive 2,4-D acid and salt toxicity values were bridged for all use scenarios to calculate RQs.
[1]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.

Table 5.4.b RQs for vascular plants based on EECs for direct application to water to represent 2,4-D acid, salt, and ester uses					
Master Label Use Category	**Model Scenario**	**Method[1]**	**Application Rate**	**Peak EEC (µg/L)**	**RQ**
Rice (acid and salts)	Direct water applications	G & A	1 app @ 1.5 lb a.e./acre	1486	**113***
Aquatic Weed Control (surface application or subsurface injection for submersed weeds) (acid and salts	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[2]	**305.34***
Aquatic Weed Control (surface application or subsurface injection for submersed weeds) (esters only)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[2]	**12.12***
Aquatic Weed Control (acid and salts)	Direct water applications	G & A	2 app @2 lb a.e./acre (30-day interval)	740	**56.49***
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @2 lb a.e./acre (30-day interval)	740	**2.2***
Aquatic Weed Control (acid and salts)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	**112.98***

Table 5.4.b RQs for vascular plants based on EECs for direct application to water to represent 2,4-D acid, salt, and ester uses					
Master Label Use Category	**Model Scenario**	**Method[1]**	**Application Rate**	**Peak EEC (µg/L)**	**RQ**
Aquatic Weed Control (esters only)	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	**4.48***

*LOC exceedances (RQ $\geq$ 1.0) are bolded. RQ (acid and salts) = use-specific peak EEC / 0.0131 mg a.e./L (E74985). RQ (esters) = use-specific peak EEC / 0.33 mg a.e./L (MRID 417352-03).
[1]G = ground application. A = aerial application.
[2]Aquatic weed control-peak water concentration: 4000 µg/L , 21-day average water concentration: 3417 µg/L, and 60-day average water concentration: 2610 µg/L. For ester direct application scenarios, 2,4-D acid input parameters were used to determine EEC. All other runoff and drift application scenarios used 2,4-D ester input parameters to determine EEC.

5.1.2 Exposures in the Terrestrial Habitat

5.1.2.1 Direct Effects to Terrestrial-phase CRLF and AW (Birds Used as Surrogate for Reptiles and Terrestrial-phase Amphibians)

As previously discussed in **Section 3.3**, potential direct effects to terrestrial species are based on foliar applications and granular applications of 2,4-D. Potential risks to birds (and, thus, reptiles and terrestrial-phase amphibians) are derived using T-REX, acute and chronic toxicity data for the most sensitive bird species for which data are available, and a variety of body-size and dietary categories.

Potential direct acute effects to the terrestrial-phase CRLF and AW due to liquid applications of 2,4-D are derived by considering dose- and dietary-based EECs modeled in T-REX for a small bird (20 g) consuming small invertebrates and acute oral and subacute dietary toxicity endpoints for avian species. Acute direct effects to the terrestrial-phase CRLF and AW via exposure to 2,4-D granules are derived based on LD_{50}/ft^2 values. Sample T-REX modeling results are located in **Appendix J.**

Potential direct chronic effects of 2,4-D to the terrestrial-phase CRLF and AW are derived by considering dietary-based exposures modeled in T-REX for birds consuming small invertebrates.

Based on the results of T-REX, all the modeled liquid applications of 2,4-D except Citrus and Potatoes exceed the Listed Species LOC for acute risks (direct effects) to the CRLF and the AW based on an acute oral basis (**Table 5.5**). The RQs that exceeded the LOC ranged from 0.39 to 38.67. For granular applications, the Listed Species LOC was exceeded with RQ values ranging from 2.43 to 130.96 (**Table 5.6.a)**. Individual effect probabilities are located in **Table 5.6.b** and are based on the highest liquid or granular application RQ. Based on the T-REX modeling results, there is one chronic LOC exceedance for 2,4-D use on aquatic weed control via surface application with an RQ of 7.58 **(Table 5.5)**.

The LC_{50} values for both the Northern bobwhite quail and mallard duck were > 3035 mg a.e./kg-diet, and no mortalities were observed at the highest test concentration (MRID 416444-02, 416444-03). Therefore, definitive acute dietary RQ values could not be derived, and the confidence in a risk call for birds is low as there were no mortalities in the dietary studies. Applying best professional judgment to this situation, considering both the unknown LC_{50} and the high uncertainty of any RQ calculated based on this non-definitive LC_{50}, it is concluded that these birds are not at risk for acute effects based on the results of the dietary studies.

All the 2,4-D modeled uses except Citrus and Potatoes have the potential to directly affect the CRLF and AW based on the acute LOC exceedances demonstrated in T-REX. Based on T-REX, the only use with chronic direct effect concerns is aquatic weed control via surface application.

Table 5.5 Upper-bound Kenega Nomogram RQs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Mammalian Prey to Liquid Applications of 2,4-D

Modeling Scenario	**Method**[1]	**Application Rate**	**RQs for CRLF and AW (Direct Effects)**[2] **and RQs for Frog Prey**[2] **(Indirect effects to CRLF and AW)**		**RQs for Mammalian Prey**[3] **(Indirect effects to CRLF and AW)**		
			Acute dose-based (Small bird consuming small insect)	**Chronic diet-based** (Bird consuming small insects)	**Acute dose-based** (Small mammal consuming short grass)	**Chronic dose-based** (Small mammal consuming short grass)	**Chronic diet- based** (Mammal consuming short grass)
Orchard Uses							
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57***	0.31	**0.52***	**45.57+**	**5.25+**
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	**0.40***	0.08	**0.13***	**11.49+**	**1.32+**
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	**0.97***	0.19	**0.32***	**28.32+**	**3.26+**
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	**1.10***	0.21	**0.36***	**31.90+**	**3.68+**
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	**1.44***	0.28	**0.47***	**41.76+**	**4.81+**
Citrus	A/G	1 app @ 0.1 lb a.e./acre	0.07	0.01	0.02	**2.08+**	0.24
Agricultural – Food Crop Uses							
Field Corn, Popcorn	A/G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre	**1.08***	0.21	**0.35***	**31.26+**	**3.60+**

Table 5.5 Upper-bound Kenega Nomogram RQs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Mammalian Prey to Liquid Applications of 2,4-D							
Modeling Scenario	Method[1]	Application Rate	RQs for CRLF and AW (Direct Effects)[2] and RQs for Frog Prey[2] (Indirect effects to CRLF and AW)		RQs for Mammalian Prey[3] (Indirect effects to CRLF and AW)		
			Acute dose-based (Small bird consuming small insect)	**Chronic diet-based** (Bird consuming small insects)	**Acute dose-based** (Small mammal consuming short grass)	**Chronic dose-based** (Small mammal consuming short grass)	**Chronic diet- based** (Mammal consuming short grass)
		April 29, 1 app @ 1.5 lb a.e./acre August 15					
Sweet Corn	A/G	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	**0.72***	0.14	**0.24***	**20.82+**	**2.40+**
Potatoes	A/G	2 apps @ 0.07 lb a.e./acre (10-day interval)	0.07	0.01	0.02	**2.12+**	0.24
Sugarcane	A/G	2 apps @ 2 lb a.e./acre (20-day interval)	**1.73***	0.34	**0.57***	**50.26+**	**5.79+**
Cereal Grains	A/G	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	**0.90***	0.18	**0.30***	**26.03+**	**3.00+**
Grain or Forage Sorghum	A/G	1 post-emergence app @ 1.0 lb a.e./acre	**0.72***	0.14	**0.24***	**20.82+**	**2.40+**
Hops	A/G	3 apps @ 0.5 lb a.e./acre (30-day interval)	**0.39***	0.08	**0.13***	**11.48+**	**1.32+**
Asparagus	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57***	0.31	**0.52***	**45.57+**	**5.25+**
Fallowland and Crop Stubble	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57***	0.31	**0.52***	**45.57+**	**5.25+**
Agricultural – Non-food Crop Uses							
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57***	0.31	**0.52***	**45.57+**	**5.25+**
Non-agricultural Uses							
Non-cropland	A/G	1 app @ 4 lb a.e./acre	**2.86***	0.56	**0.94***	**83.29+**	**9.60+**
Forestry	A/G	1 app @ 4 lb a.e./acre	**2.86***	0.56	**0.94***	**83.29+**	**9.60+**
Tree and Brush	A/G	1 app @ 4 lb a.e./acre	**2.86***	0.56	**0.94***	**83.29+**	**9.60+**

Table 5.5 Upper-bound Kenega Nomogram RQs for Dietary-and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Mammalian Prey to Liquid Applications of 2,4-D							
Modeling Scenario	Method[1]	Application Rate	RQs for CRLF and AW (Direct Effects)[2] and RQs for Frog Prey[2] (Indirect effects to CRLF and AW)		RQs for Mammalian Prey[3] (Indirect effects to CRLF and AW)		
			Acute dose-based (Small bird consuming small insect)	**Chronic diet-based** (Bird consuming small insects)	**Acute dose-based** (Small mammal consuming short grass)	**Chronic dose-based** (Small mammal consuming short grass)	**Chronic diet- based** (Mammal consuming short grass)
Control							
Ornamental Turf	A/G	2 apps @ 1.5 lb a.e./acre (21-day interval)	**1.28***	0.25	**0.42***	**37.21+**	**4.29+**
Grass Grown for Seed and Sod	A/G	2 apps @ 2 lb a.e./acre (21-day interval)	**1.71***	0.33	**0.56***	**49.61+**	**5.72+**
Direct Application to Water Uses							
Rice	A/G	1 app @ 1.5 lb a.e./acre	**1.07***	0.21	**0.35***	**31.23+**	**3.60+**
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre-foot[5]	**38.67***	**7.58+**	**12.75***	**1124.41+**	**129.60+**
Aquatic Weed Control	A/G	2 app @ 2 lb a.e./acre (30-day interval)	**1.57***	0.31	**0.52***	**45.57+**	**5.25+**
Aquatic Weed Control	A/G	2 app @ 4 lb a.e./acre (21-day interval)	**3.41***	0.67	**1.12***	**99.22+**	**11.44+**

[1]G = ground application. A = aerial application.
[2]EECs based on small bird (20 g), which consumes small insects.
[3]EECs based on small mammal (15 g), which consumes short grass.
[4]These EECs also apply for terrestrial invertebrates (small insects).
[5]Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre
*Acute (RQ ≥ 0.1) exceeds acute endangered species level of concern (LOC).
+Chronic (RQ ≥ 1.0) exceeds chronic level of concern (LOC).

Table 5.6.a Acute RQs for Granular Applications of 2,4-D for (1) Direct Effects on the Terrestrial-phase CRLF and AW, (2) Indirect Effects on the AW (birds as prey), and (3) Indirect Effects on the Terrestrial-phase CRLF and AW (frogs are prey)			
Scenario	Application Rate	EEC (mg a.e./ft^2)	Acute RQ (LD_{50})[1]
Agricultural Food Crop Uses			
Field Corn, Popcorn	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	15.62	**3.64***
Sweet Corn	1 app @ 1 lb a.e./acre March 15; 1 app @ 0.5 lb a.e./acre April 29	10.41	**2.43***
Grain or Forage Sorghum	1 post-emergence app @ 1.0 lb a.e./acre	10.41	**2.43***

Table 5.6.a Acute RQs for Granular Applications of 2,4-D for (1) Direct Effects on the Terrestrial-phase CRLF and AW, (2) Indirect Effects on the AW (birds as prey), and (3) Indirect Effects on the Terrestrial-phase CRLF and AW (frogs are prey)			
Scenario	**Application Rate**	**EEC (mg a.e./ft^2)**	**Acute RQ (LD_{50})[1]**
Non-Agricultural Uses			
Non-cropland	1 app @ 4 lb a.e./acre	41.65	**9.70***
Ornamental Turf	2 apps @ 1.5 lb a.e./acre (21-day interval)	15.62	**3.64***
Grass Grown for Seed and Sod	2 apps @ 2 lb a.e./acre (21-day interval)	20.83	**4.85***
Direct Application to Water Uses			
Aquatic Weed Control	1 app @ 10.8 lb a.e./acre-foot	562.30	**130.96***
Aquatic Weed Control	2 app @ 2 lb a.e./acre (30-day interval)	20.83	**4.85***
Aquatic Weed Control	2 app @ 4 lb a.e./acre (21-day interval)	41.65	**9.70***

[1]Calculation based on Northern bobwhite quail acute oral dose LD_{50} =298 mg a.e./kg-bw (MRID 442757-01).
*Acute RQ ≥ 0.1 exceeds acute listed species level of concern (LOC).

Table 5.6.b Summary of Direct Effect RQs for the Terrestrial–phase CRLF , Individual Effect Probabilities (based on direct effect acute RQs presented in Tables 5.5 and 5.6a)				
Master Label Use Category	**Application Type[1]**	**Application Rate (interval between applications)**	**Highest Dose –Based RQ**	**Probability of Individual Effect at RQ[2]**
Orchard Uses				
Nut Orchards, Pistachios	Liquid	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57**	1 in 1.23E+00
Filberts	Liquid	4 apps @ 0.5 lb a.e./acre[3] (30-day interval)	**0.40**	1 in 2.73E+01
Grapes (all)	Liquid	1 app @ 1.36 lb a.e./acre	**0.97**	1 in 2.10E+00
Blueberries	Liquid	2 apps @ 1.4 lb a.e./acre (30-day interval)	**1.10**	1 in 1.74E+00
Stone and Pome Fruits	Liquid	2 apps @ 2 lb a.e./acre (75-day interval)	**1.44**	1 in 1.66E+00
Citrus	Liquid	1 app @ 0.1 lb a.e./acre	No LOC exceedance	
Agricultural – Food Crop Uses				
Field Corn, Popcorn	Granular	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29 1 app @ 1.5 lb a.e./acre August 15	**2.43**	1 in 1.04E+00
Sweet Corn	Granular	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	**2.43**	1 in 1.04E+00

Table 5.6.b Summary of Direct Effect RQs for the Terrestrial–phase CRLF , Individual Effect Probabilities (based on direct effect acute RQs presented in Tables 5.5 and 5.6a)				
Master Label Use Category	**Application Type**[1]	**Application Rate (interval between applications)**	**Highest Dose –Based RQ**	**Probability of Individual Effect at RQ**[2]
		(45-day interval)		
Potatoes	Liquid	2 apps @ 0.07 lb a.e./acre (10-day interval)	No LOC exceedance	
Sugarcane	Liquid	2 app @ 2 lb a.e./acre (20-day interval)	**1.73**	1 in 1.17E+00
Cereal Grains	Liquid	1 post-emergence app @ 1.25 lb a.e./acre and 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	**0.90**	1 in 2.39E+00
Grain or Forage Sorghum	Granular	1 post-emergence app @ 1.0 lb a.e./acre	**2.43**	1 in 1.04E+00
Hops	Liquid	3 apps @ 0.5 lb a.e./acre (30-day interval)	**0.39**	1 in 3.04E+01
Asparagus	Liquid	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57**	1 in 1.23E+00
Fallowland and Crop Stubble	Liquid	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57**	1 in 1.23E+00
Agricultural – Non-food Crop Uses				
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	Liquid	2 apps @ 2 lb a.e./acre (30-day interval)	**1.57**	1 in 1.23E+00
Non-agricultural Uses				
Non-cropland	Granular	1 app @ 4 lb a.e./acre	**9.70**	1 in 1.00E+00
Forestry	Liquid	1 app @ 4 lb a.e./acre	**2.86**	1 in 1.00E+00
Tree and Brush Control	Liquid	1 app @ 4 lb a.e./acre	**2.86**	1 in 1.00E+00
Ornamental Turf	Granular	2 apps @ 1.5 lb a.e./acre (21-day interval)	**3.64**	1 in 1.01E+00
Grass Grown for Seed and Sod	Granular	2 apps @ 2 lb a.e./acre (21-day interval)	**4.85**	1 in 1.00E+00
Direct Application to Water Uses				
Rice	Liquid	1 app @ 1.5 lb a.e./acre	**1.07**	1 in 1.81E+00
Aquatic Weed	Granular	1 app @ 10.8 lb a.e./acre-	**130.96**	1 in 1.00E+00

Table 5.6.b Summary of Direct Effect RQs for the Terrestrial–phase CRLF , Individual Effect Probabilities (based on direct effect acute RQs presented in Tables 5.5 and 5.6a)				
Master Label Use Category	**Application Type[1]**	**Application Rate (interval between applications)**	**Highest Dose –Based RQ**	**Probability of Individual Effect at RQ[2]**
Control Surface application or subsurface injection		foot		
Aquatic Weed Control Ditchbank	Granular	2 apps @ 2 lb a.e./acre (30-day interval)	**4.85**	1 in 1.00E+00
Aquatic Weed Control Surface application	Granular	2 app @ 4 lb a.e./acre (21-day interval)	**9.70**	1 in 1.00E+00
[1]Liquid or granular, application type listed provided the highest dose-based RQ for each use scenario for "small birds consuming small insects" [2]A slope value was not available for the acute bird of LD_{50} = 298 mg a.e. /kg-bw bobwhite quail (MRID 442757-01), therefore the probability was calculated based on the default slope value of 4.5.				

5.1.2.2 Indirect Effects to Terrestrial-phase CRLF and AW via Reduction in Prey (Mammals, Birds, Terrestrial invertebrates, and Frogs)

5.1.2.2.1 Mammals

Potential risks to mammals are derived using T-REX, acute and chronic rat toxicity data, and a variety of body-size and dietary categories.

The T-REX Modeling results for liquid applications are presented in **Table 5.5**. Based on the T-REX results, all the modeled uses exceed the Agency LOC for acute and chronic risk to mammals. The acute RQs range from 0.13 to 12.75, and the chronic RQs range from 1.32 to 1112.41. Indirect effects to terrestrial-phase CRLFs and AW via ingestion of small mammals that may consume 2,4-D granules are based on LD_{50}/ft^2 values. The Listed Species LOC was exceeded with RQ values ranging from 0.72 to 38.68 for granular applications of 2,4-D (**Table 5.7**).

Since the acute and chronic RQs are exceeded, there is a potential for indirect effects to those listed species that rely on mammals during at least some portion of their life-cycle (*i.e.*, CRLF and AW through mammalian prey consumption).

Table 5.7 Acute RQs used to Estimate Indirect effects to terrestrial-phase CRLFs and AW via ingestion of small mammals that may consume 2,4-D granules			
Scenario	**Application Rate**	**EEC[1] (mg a.e./ft^2)**	**Acute RQ[2]**
Agricultural Food Crop Uses			
Field Corn, Popcorn	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	15.62	**1.07***
Sweet Corn	1 app @ 1 lb a.e./acre March 15; 1 app @ 0.5 lb a.e./acre April 29	10.41	**0.72***
Grain or Forage Sorghum	1 post-emergence app @ 1.0 lb a.e./acre	10.41	**0.72***
Non-Agricultural Uses			
Non-cropland	1 app @ 4 lb a.e./acre	41.65	**2.86***
Ornamental Turf	2 apps @ 1.5 lb a.e./acre (21-day interval)	15.62	**1.07***
Grass Grown for Seed and Sod	2 apps @ 2 lb a.e./acre (21-day interval)	20.83	**1.43***
Direct Application to Water Uses			
Aquatic Weed Control	1 app @ 10.8 lb a.e./acre foot	562.30	**38.68***
Aquatic Weed Control	2 app @ 2 lb a.e./acre (30-day interval)	20.83	**1.43***
Aquatic Weed Control	2 app @ 4 lb a.e./acre (21-day interval)	41.65	**2.86***

[1]EEC based on soil concentration.
[2]Calculation based on rat acute oral dose LD_{50} =441mg a.e./kg-bw (MRID 414135-01).
*Acute RQ ≥ 0.1 exceeds acute listed species level of concern (LOC).

5.1.2.2.2 Birds (Assessed for AW Only)

An additional prey item of the AW is small birds. In order to assess risks to these organisms, dietary-based and dose-based exposures modeled in T-REX for a small bird (20 g) consuming short grass are used for non-granular applications to estimate acute and chronic risks (**Table 5.8).** Acute risks for granular applications are estimated in **Table 5.6.a.** Since the acute and chronic RQs are exceeded, there is a potential for indirect effects to AW as they rely on avian prey during at least some portion of their life-cycle.

Table 5.8 Upper-bound Kenega Nomogram EECs for Dietary- and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D						
Modeling Scenario	Method[1]	Application Rate	RQs for Avian Prey (Indirect Effects to AW)[2]		RQs for Terrestrial Invertebrate Prey (Indirect Effects to CRLF and AW)	
			Acute dose-based (Small bird consuming short grass)	**Chronic dietary-based** (Bird consuming short grass)	**Small Invertebrates**	**Large Invertebrates**
Orchard Uses						
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	**2.79***	0.55	**0.57****	**0.06****
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	**0.70***	0.14	**0.15****	0.02
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	**1.73***	0.34	**0.35****	0.04
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	**1.95***	0.38	**0.40****	**0.05****
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	**2.55***	0.50	**0.53****	**0.06****
Citrus	A/G	1 app @ 0.1 lb a.e./acre	**0.13***	0.02	0.03	0.004
Agricultural – Food Crop Uses						
Field Corn, Popcorn	A/G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	**1.91***	0.37	**0.39****	**0.05****
Sweet Corn	A/G	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	**1.27***	0.25	**0.26****	0.03
Potatoes	A/G	2 apps @ 0.07 lb a.e./acre (10-day interval)	**0.13***	0.03	0.03	0.004
Sugarcane	A/G	2 apps @ 2 lb a.e./acre (20-day interval)	**3.07***	0.60	**0.63****	**0.07****
Cereal Grains	A/G	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	**1.59***	0.31	**0.33****	0.04
Grain or Forage Sorghum	A/G	1 post-emergence app @ 1.0 lb a.e./acre	**1.27***	0.25	**0.26****	0.03
Hops	A/G	3 apps @ 0.5 lb a.e./acre (30-day interval)	**0.70***	0.14	**0.15****	0.02
Asparagus	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	**2.79***	0.55	**0.57****	**0.06****
Fallowland and Crop Stubble	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	**2.79***	0.55	**0.57****	**0.06****

Table 5.8 Upper-bound Kenega Nomogram EECs for Dietary- and Dose-based Exposures of the Terrestrial-phase CRLF and AW and its Prey to Liquid Applications of 2,4-D						
			RQs for Avian Prey (Indirect Effects to AW)[2]		RQs for Terrestrial Invertebrate Prey (Indirect Effects to CRLF and AW)	
Modeling Scenario	**Method[1]**	**Application Rate**	**Acute dose-based** (Small bird consuming short grass)	**Chronic dietary-based** (Bird consuming short grass)	**Small Invertebrates**	**Large Invertebrates**
Agricultural – Non-food Crop Uses						
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	**2.79***	0.55	**0.57****	**0.06****
Non-agricultural Uses						
Non-cropland	A/G	1 app @ 4 lb a.e./acre	**5.09***	**1.00+**	**1.05****	**0.12****
Forestry	A/G	1 app @ 4 lb a.e./acre	**5.09***	**1.00+**	**1.05****	**0.12****
Tree and Brush Control	A/G	1 app @ 4 lb a.e./acre	**5.09***	**1.00+**	**1.05****	**0.12****
Ornamental Turf	A/G	2 apps @ 1.5 lb a.e./acre (21-day interval)	**2.28***	0.45	**0.47***	**0.05****
Grass Grown for Seed and Sod	A/G	2 apps @ 2 lb a.e./acre (21-day interval)	**3.03***	0.59	**0.63****	**0.07****
Direct Application to Water Uses						
Rice	A/G	1 app @ 1.5 lb a.e./acre	**1.91***	0.37	**0.39****	**0.05****
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[3]	**68.75***	**13.47+**	**14.16****	**1.57****
Aquatic Weed Control	A/G	2 app @ 2 lb a.e./acre (30-day interval)	**2.79***	0.55	**0.57****	**0.06****
Aquatic Weed Control	A/G	2 app @ 4 lb a.e./acre (21-day interval)	**6.07***	**1.19+**	**1.24****	**0.14****

[1]G = ground application. A = aerial application.
[2]EECs based on small bird (20 g) which consumes short grass.
[3]Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre
*Acute RQ ≥ 0.1 exceeds acute listed species level of concern (LOC) for birds.
**Acute RQ ≥ 0.05 exceeds acute level of concern (LOC) for terrestrial invertebrates.
+Chronic RQ ≥ 1.0 exceeds chronic level of concern (LOC) for birds.

5.1.2.2.3 Terrestrial Invertebrates

In order to assess the risks of 2,4-D to terrestrial invertebrates, the honey bee is used as a surrogate for terrestrial invertebrates. The toxicity value for terrestrial invertebrates is calculated by multiplying the lowest available acute contact LD_{50} of 66 µg a.e./bee by 1 bee/0.128g, which is based on the weight of an adult honey bee. EECs (µg a.e./g of bee) calculated by T-REX for small and large insects are divided by the calculated toxicity value for terrestrial invertebrates, which is 515 mg a.e./kg-insect. It is important to note that the calculated RQs may overestimate risk as the LD_{50} values from all submitted bee studies were non-definitive (50% mortality was not reached at the highest dose). The results of the bee RQs are tabulated in **Table 5.8**.

Based on the bee RQ calculations, all of the modeled uses of 2,4-D except citrus and potatoes exceed the LOC for acute risk to small terrestrial invertebrates with RQs ranging from 0.15 to 14.16. For large invertebrates, all uses except filberts, grapes, citrus, sweet corn, potatoes, cereal grains, grain or forage sorghum, and hops exceed the LOC with RQs ranging between 0.05 to 1.57 (**Table 5.8**).

5.1.2.2.4 Frogs

An additional prey item of the adult terrestrial-phase CRLF and AW is other species of frogs. In order to assess risks to these organisms, dietary-based and dose-based exposures modeled in T-REX for a small bird (20 g) consuming small invertebrates are used for non-granular applications to estimate acute and chronic risks. Acute and chronic risks for liquid applications are estimated in **Table 5.5**, and acute risks for granular applications are estimated in **Table 5.6.a.** These acute and chronic LOC exceedances indicate that there is a potential for indirect effects to those listed species that rely on birds (and, thus, reptiles and/or terrestrial-phase amphibians) during at least some portion of their life-cycle (*i.e.*, CRLF and AW).

5.1.2.3 Indirect Effects to Terrestrial-phase CRLF and AW via Reduction in Terrestrial Plant Community (Riparian and Upland Habitat)

Potential indirect effects to the CRLF resulting from direct effects on riparian and upland vegetation are assessed using RQs from terrestrial plant seedling emergence and vegetative vigor EC_{25} data as a screen. Based on the TerrPlant modeling results, there are LOC exceedances for all modeled uses except for citrus and potatoes for risks to non-listed monocot plants; all modeled uses result in LOC exceedances for all non-listed dicot plants. RQs that exceed the LOC range from 2.63 to 22.68 for monocots and 2.98 to 183.33 for dicots **(Tables 5.9.a and 5.9.b)**. Since the non-listed plant LOCs are exceeded, there is potential for indirect effects to those listed species that rely on terrestrial plants during at least some portion of their life-cycle (*i.e.*, CRLF and AW).

Table 5.9.a TerrPlant RQs for Monocots Inhabiting Dry and Semi-aquatic Areas Exposed to 2,4-D via Runoff and Drift (single application only)						
Modeling Scenario	**Method[1]**	**Application Rate**	**Drift Value (%)**	**Dry Area RQ**	**Semi-aquatic Area RQ**	**Spray Drift RQ**
Orchard Uses						
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	1	**1.24***	**10.52***	0.23
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	1	0.31	**2.63***	<0.1
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	1	0.84	**7.15***	0.15
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	1	0.87	**7.36***	0.16
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	1	**1.24***	**10.52***	0.23
Citrus	A	1 app @ 0.1 lb a.e./acre	5	0.10	0.57	<0.1
	G		1	<0.1	0.53	<0.1
Agricultural – Food Crop Uses						
Field Corn, Popcorn	A	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	5	**1.55***	**8.51***	0.85
	G		1	0.93	**7.89***	0.17
Sweet Corn	A	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	5	**1.03***	**5.67***	0.57
	G		1	0.62	**5.26***	0.11
Potatoes	A	2 apps @ 0.07 lb a.e./acre (10-day interval)	5	<0.1	0.40	<0.1
	G		1	<0.1	0.37	<0.1
Sugarcane	A	2 apps @ 2 lb a.e./acre (20-day interval)	5	**2.06***	**11.34***	**1.14***
	G		1	**1.24***	**10.52***	0.23
Cereal Grains	A	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	5	**1.29***	**7.09***	0.71
	G		1	0.77	**6.57***	0.14
Grain or Forage Sorghum	A	1 post-emergence app @ 1.0 lb a.e./acre	5	**1.03***	**5.67***	0.57
	G		1	0.62	**5.26***	0.11
Hops	A	3 apps @ 0.5 lb a.e./acre (30-day interval)	5	0.52	**2.84***	0.28
	G		1	0.31	**2.63***	<0.1
Asparagus	A	2 apps @ 2 lb a.e./acre (30-day interval)	5	**2.06***	**11.34***	**1.14***
	G		1	**1.24 ***	**10.52***	0.23
Fallowland and Crop Stubble	A	2 apps @ 2 lb a.e./acre (30-day interval)	5	**2.06***	**11.34***	**1.14***
	G		1	**1.24 ***	**10.52***	0.23
Agricultural – Non-food Crop Uses						
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural	G	2 apps @ 2 lb a.e./acre (30-day interval)	1	**1.24***	**10.52***	0.23

Table 5.9.a TerrPlant RQs for Monocots Inhabiting Dry and Semi-aquatic Areas Exposed to 2,4-D via Runoff and Drift (single application only)						
Modeling Scenario	**Method[1]**	**Application Rate**	**Drift Value (%)**	**Dry Area RQ**	**Semi-aquatic Area RQ**	**Spray Drift RQ**
Production						
Non-agricultural Uses						
Non-cropland	A	1 app @ 4 lb a.e./acre	5	**4.12***	**22.68***	**2.27***
	G		1	**2.47***	**21.03***	0.45
Forestry	A	1 app @ 4 lb a.e./acre	5	**4.12***	**22.68***	**2.27***
	G		1	**2.47***	**21.03***	0.45
Tree and Brush Control	A	1 app @ 4 lb a.e./acre	5	**4.12***	**22.68***	**2.27***
	G		1	**2.47***	**21.03***	0.45
Ornamental Turf	A	2 apps @ 1.5 lb a.e./acre (21-day interval)	5	**1.55***	**8.51***	0.85
	G		1	0.93	**7.89***	0.17
Grass Grown for Seed and Sod	A	2 apps @ 2 lb a.e./acre (21-day interval)	5	**2.06***	**11.34***	**1.14***
	G		1	**1.24***	**10.52***	0.23
Direct Application to Water Uses						
Rice	A	1 app @ 1.5 lb a.e./acre	5	**1.55***	**8.51***	0.85
	G		1	0.93	**7.89***	0.17

[1]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.
[2]EECs calculated based on a single application. If crop labeled for multiple applications within a year, the highest single rate was used.
*RQ ≥ 1.0 exceeds non-listed level of concern (LOC).

Table 5.9.b TerrPlant RQs for Dicots Inhabiting Dry and Semi-aquatic Areas Exposed to 2,4-D via Runoff and Drift (single application only)						
Modeling Scenario	**Method[1]**	**Application Rate**	**Drift Value (%)**	**Dry Area RQ**	**Semi-aquatic Area RQ**	**Spray Drift RQ**
Orchard Uses						
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	1	**10.00***	**85.00***	**9.52***
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	1	**2.50***	**21.25***	**2.38***
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	1	**6.80***	**57.80***	**6.48***
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	1	**7.00***	**59.50***	**6.67***
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	1	**10.00 ***	**85.00***	**9.52***

Table 5.9.b TerrPlant RQs for Dicots Inhabiting Dry and Semi-aquatic Areas Exposed to 2,4-D via Runoff and Drift (single application only)						
Modeling Scenario	**Method[1]**	**Application Rate**	**Drift Value (%)**	**Dry Area RQ**	**Semi-aquatic Area RQ**	**Spray Drift RQ**
Citrus	A	1 app @ 0.1 lb a.e./acre	5	0.83	**4.58***	**2.38***
	G		1	0.50	**4.25***	0.48
Agricultural – Food Crop Uses						
Field Corn, Popcorn	A	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	5	**12.50***	**68.75***	**35.71***
	G		1	**7.50***	**63.75***	**7.14***
Sweet Corn	A	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	5	**8.33***	**45.83***	**23.81***
	G		1	**5.00***	**42.50***	**4.76***
Potatoes	A	2 apps @ 0.07 lb a.e./acre (10-day interval)	5	0.58	**3.21***	**1.67***
	G		1	0.35	**2.98***	0.33
Sugarcane	A	2 apps @ 2 lb a.e./acre (20-day interval)	5	**16.67***	**91.67***	**47.62***
	G		1	**10.00***	**85.00***	**9.52***
Cereal Grains	A	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	5	**10.42***	**57.29***	**29.76***
	G		1	**6.25***	**53.13***	**5.95***
Grain or Forage Sorghum	A	1 post-emergence app @ 1.0 lb a.e./acre	5	**8.33***	**45.83***	**23.81***
	G		1	**5.00***	**42.50***	**4.76***
Hops	A	3 apps @ 0.5 lb a.e./acre (30-day interval)	5	**4.17***	**22.92***	**11.90***
	G		1	**2.50***	**21.25***	**2.38***
Asparagus	A	2 apps @ 2 lb a.e./acre (30-day interval)	5	**16.67***	**91.67***	**47.62***
	G		1	**10.00 ***	**85.00***	**9.52***
Fallowland and Crop Stubble	A	2 apps @ 2 lb a.e./acre (30-day interval)	5	**16.67***	**91.67***	**47.62***
	G		1	**10.00 ***	**85.00***	**9.52***
Agricultural – Non-food Crop Uses						
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	1	**10.00 ***	**85.00***	**9.52***
Non-agricultural Uses						

Table 5.9.b TerrPlant RQs for Dicots Inhabiting Dry and Semi-aquatic Areas Exposed to 2,4-D via Runoff and Drift (single application only)						
Modeling Scenario	**Method[1]**	**Application Rate**	**Drift Value (%)**	**Dry Area RQ**	**Semi-aquatic Area RQ**	**Spray Drift RQ**
Non-cropland	A	1 app @ 4 lb a.e./acre	5	**33.33***	**183.33***	**95.24***
	G		1	**20.00***	**170.00***	**19.05***
Forestry	A	1 app @ 4 lb a.e./acre	5	**33.33***	**183.33***	**95.24***
	G		1	**20.00***	**170.00***	**19.05***
Tree and Brush Control	A	1 app @ 4 lb a.e./acre	5	**33.33***	**183.33***	**95.24***
	G		1	**20.00***	**170.00***	**19.05***
Ornamental Turf	A	2 apps @ 1.5 lb a.e./acre (21-day interval)	5	**12.50***	**68.75***	**35.71***
	G		1	**7.50***	**63.75***	**7.14***
Grass Grown for Seed and Sod	A	2 apps @ 2 lb a.e./acre (21-day interval)	5	**16.67**	**91.67***	**47.62***
	G		1	**10.00***	**85.00***	**9.52***
Direct Application to Water Uses						
Rice	A	1 app @ 1.5 lb a.e./acre	5	**12.50***	**68.75***	**35.71***
	G		1	**7.50***	**63.75***	**7.14***

[1]G = ground application. A = aerial application. All applications are liquid unless otherwise specified.
[2]EECs calculated based on a single application. If crop labeled for multiple applications within a year, the highest single rate was used.
*RQ ≥ 1.0 exceeds non-listed level of concern (LOC).

5.1.3 Primary Constituent Elements of Designated Critical Habitat

For 2,4-D use, the assessment endpoints for designated critical habitat PCEs involve the same endpoints as those being assessed relative to the potential for direct and indirect effects to the listed species assessed here. Therefore, the effects determinations for direct and indirect effects are used as the basis of the effects determination for potential modification to designated critical habitat. The potential for effects on critical habitat PCEs are discussed in **Section 5.2.4**.

5.2 Risk Description

The risk description synthesizes an overall conclusion regarding the likelihood of adverse impacts leading to an effects determination (*i.e.*, "no effect," "may affect but not likely to adversely affect," or "may affect and likely to adversely affect") for the CRLF and the AW and their designated critical habitats. If the RQs presented in the Risk Estimation (**Section 5.1**) show no direct or indirect effects for the assessed species, and no modification to PCEs of the designated critical habitat, a "no effect" determination is made, based on 2,4-D's use within the action area. However, if LOCs for direct or indirect effect are exceeded or effects may modify the PCEs of the critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding 2,4-D.

Based on the RQ results from the direct and indirect risk estimation for 2,4 D, a preliminary effects determination for the CRLF and the AW is "may affect." A summary of the risk estimation results are provided in **Table 5.10.a and 5.11.a** for direct and indirect effects to the listed species assessed here and in **Table 5.10.b and 5.11.b** for the PCEs of their designated critical habitat.

Table 5.10.a Risk Estimation Summary for 2,4-D - Direct and Indirect Effects to CRLF

Assessment Endpoint	LOC Exceedances (Y/N)	Description of Results of Risk Estimation
Aquatic Phase (eggs, larvae, tadpoles, juveniles, and adults)		
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases.	Yes	*Survival*: LOC was exceeded in the aerial Forestry, Tree and Brush Control runoff and drift ester uses and all direct application to water scenarios **(Table 5.1.a, 5.1.b, and 5.1.c**). *Growth and reproduction:* Chronic LOC was not exceeded for any scenarios **(Table 5.1.a and 5.1.b**).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.,* freshwater invertebrates, non-vascular plants).	Yes	*Freshwater fish:* Listed Species LOC was exceeded in the aerial Forestry, Tree and Brush Control runoff and drift ester uses and all direct application to water scenarios, no Chronic LOCs exceeded **(Table 5.1.a, 5.1.b, and 5.1.c**). *Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios **(Table 5.2**). *Freshwater invertebrates*: Acute LOC was exceeded for all direct application to water scenarios **(Table 5.3.a and 5.3.b**).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat, cover, and/or primary productivity (*i.e.,* aquatic plant community).	Yes	*Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios **(Table 5.2**). *Vascular aquatic plants*: LOC was exceeded for several acid/salt use scenarios and all direct application to water scenarios (**Table 5.4.a and 5.4.b**).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. (**Tables 5.9.a and 5.9.b**).
Terrestrial Phase (Juveniles and adults)		

Table 5.10.a Risk Estimation Summary for 2,4-D - Direct and Indirect Effects to CRLF		
Assessment Endpoint	**LOC Exceedances (Y/N)**	**Description of Results of Risk Estimation**
Direct Effects Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles.	Yes	*Survival*: Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for granular applications (**Tables 5.5, 5.6.a, and 5.6.b**). *Growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbanks) for liquid application (**Table 5.5**).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.,* terrestrial invertebrates, small terrestrial mammals and terrestrial phase amphibians).	Yes	*Terrestrial invertebrates*: Acute LOC for small insects was exceeded for all scenarios except citrus and potatoes. Acute LOC for large insects was exceeded for several scenarios. (**Table 5.8**). *Terrestrial-phase amphibians*: Acute LOCs were exceeded in all T-REX modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for T-REX modeled granular applications (**Table 5.5 and 5.6.a**). *Small terrestrial mammals*: Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for granular applications (**Table 5.5 and 5.7**).
Indirect Effects Survival, growth, and reproduction of CRLF individuals via effects on habitat (*i.e.,* riparian vegetation).	Yes	*Terrestrial plants:* LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).

Table 5.10.b Risk Estimation Summary for 2,4-D – PCEs of Designated Critical Habitat for the CRLF[1]		
Assessment Endpoint	**Habitat Modification (Y/N)**	**Description of Results of Risk Estimation**
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	Yes	*Freshwater fish*: Acute LOC was exceeded for aerial Forestry and Tree and Brush Control all direct application to water scenarios (**Table 5.1.a, 5.1.b, and 5.1.c**). *Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios (**Table 5.2**). *Freshwater invertebrate:* Acute LOC was exceeded for all direct application to water scenarios (**Table 5.3.a and 5.3.b**).
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae).	Yes	*Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios (**Table 5.2**).
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance.	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).

Table 5.10.b Risk Estimation Summary for 2,4-D – PCEs of Designated Critical Habitat for the CRLF[1]		
Assessment Endpoint	**Habitat Modification (Y/N)**	**Description of Results of Risk Estimation**
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).
Reduction and/or modification of food sources for terrestrial phase juveniles and adults.	Yes	*Terrestrial food sources*: Based on likely effects to small mammals, amphibians, and terrestrial invertebrates, reduction in food sources is expected (**Tables 5.5**, **5.6.a**, **5.7**, **5.8**).
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	Yes	*Terrestrial food sources*: Based on likely effects to small mammals, amphibians, and terrestrial invertebrates, reduction in food sources is expected (**Tables 5.5**, **5.6.a**, **5.7**, **5.8**).
[1]These PCEs are in addition to more general requirements for habitat areas that provide essential life cycle needs of the species such as, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species.		

Table 5.11.a Risk Estimation Summary for 2,4-D - Direct and Indirect Effects to the AW		
Assessment Endpoint	**Habitat Modification (Y/N)**	**Description of Results of Risk Estimation**
Terrestrial Phase (Juveniles and adults)		
Direct Effects Survival, growth, and reproduction of AW individuals via direct effects on terrestrial phase adults and juveniles.	Yes	*Survival*: Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for granular applications (**Tables 5.5, 5.6.a, and 5.6.b**). *Growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbanks) for liquid application (**Table 5.5**).

Table 5.11.a Risk Estimation Summary for 2,4-D - Direct and Indirect Effects to the AW		
Assessment Endpoint	**Habitat Modification (Y/N)**	**Description of Results of Risk Estimation**
Indirect Effects Survival, growth, and reproduction of AW individuals via effects on prey (*i.e.,* terrestrial invertebrates, small terrestrial mammals and terrestrial phase amphibians).	Yes	*Terrestrial invertebrates*: Acute LOC for small insects was exceeded for all scenarios except citrus and potatoes. Acute LOC for large insects was exceeded for several scenarios. (**Table 5.8**). *Terrestrial-phase amphibians*: Acute LOCs were exceeded in all T-REX modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for T-REX modeled granular applications (**Table 5.5 and 5.6.a**). *Small terrestrial mammals*: Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for granular applications (**Table 5.5 and 5.7**). *Small birds:* Acute LOC exceeded in all modeled scenarios **(Table 5.6a and 5.8).**
Indirect Effects Survival, growth, and reproduction of AW individuals via effects on habitat (*i.e.,* riparian vegetation).	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).

Table 5.11.b Risk Estimation Summary for 2,4-D – PCEs of Designated Critical Habitat for the AW[1]		
Assessment Endpoint	**LOC Exceedances (Y/N)**	**Description of Results of Risk Estimation**
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Scrub/shrub communities with a mosaic of open and closed canopy	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).
Woodland or annual grassland plant communities contiguous to lands containing scrub/shrub communities with a mosaic of open and closed canopy	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).
Lands containing rock outcrops, talus, and small mammal burrows within or adjacent to 1) scrub/shrub communities with a mosaic of open and closed canopy and/or 2) woodland or annual grassland plant communities contiguous to lands containing scrub/shrub communities with a mosaic of open and closed canopy	Yes	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios (**Tables 5.9.a and 5.9.b**).
[1]These PCEs are in addition to more general requirements for habitat areas that provide essential life cycle needs of the species such as, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species.		

Following this "may affect" determination, additional information will be considered to refine the potential for exposure at the predicted levels based on the life history characteristics (*i.e.*, habitat range, feeding preferences, *etc.*) of the CRLF and the AW. Based on the best available information, the Agency uses the refined evaluation to distinguish those actions that "may affect but are not likely to adversely affect" from those actions that "may affect and are likely to adversely affect" the CRLF and the AW and their designated critical habitats.

The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the CRLF and the AW and their designated critical habitats include the following:

- Significance of Effect: Insignificant effects are those that cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where "take" occurs for even a single individual. "Take" in this context means to harass or harm, defined as the following:

- Harm includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.
- Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.

- Likelihood of the Effect Occurring: Discountable effects are those that are extremely unlikely to occur.
- Adverse Nature of Effect: Effects that are wholly beneficial without any adverse effects are not considered adverse.

A description of the risk and effects determination for each of the established assessment endpoints for the CRLF and the AW and their designated critical habitats is provided in **Sections 5.2.1** through **5.2.3**.

As previously discussed, the results of this analysis lead to a preliminary "may affect" determination for the CRLF and the AW based on labeled 2,4-D usage in California due to the large number of LOC exceedances across multiple taxonomic groups and multiple cropping scenarios.

For both the CRLF and the AW, this "may affect" determination is refined to a "likely to adversely affect" (LAA) determination for all labeled crops except citrus and potatoes based on the characterization of potential effects and likelihood of exposure discussed below. Citrus and potato 2,4-D use is refined to a "may affect, not likely to adversely affect" (NLAA) determination.

5.2.1 Direct Effects

5.2.1.1 Aquatic-Phase CRLF

The aquatic-phase considers life stages of the frog that are obligatory aquatic organisms, including eggs and larvae. It also considers submerged terrestrial-phase juveniles and adults, which spend a portion of their time in water bodies that may receive runoff and spray drift containing 2,4-D.

LOC exceedances:
Of the scenarios modeled, acute listed species LOCs were exceeded in the aerial Forestry and Tree and Brush Control uses as well as all of the direct application to water uses (Rice and Aquatic Weed Control). Exceeding RQs ranged from 0.05 to 15.38. There were no chronic LOC exceedances.

Comparison of modeled to observed water concentrations:
The available monitoring data were presented in **Section 3.2.7**. Compared with the modeling results, the modeled values are much higher than USGS NAWQA and CDPR data for California. The lack of agreement between the model and monitoring results is

not unexpected since the monitoring data were not designed to target areas of 2,4-D usage. With the exception of direct aquatic applications such as rice use and aquatic weed control, the model predictions are comparable with the available registrant-submitted fate studies.

Analysis of aquatic-phase amphibian data:
As previously discussed in the aquatic toxicity portion of this assessment (**Section 4.2**), aquatic amphibian data were submitted by the registrant and available in the open literature. However, since it was less sensitive than the freshwater fish data and it is unknown where the CRLF falls on sensitivity distribution for aquatic-phase amphibians or on a sensitivity distribution for aquatic invertebrates, EFED determined that using the most sensitive aquatic vertebrate toxicity data would be appropriate. However, even if the reviewed frog data were used to calculate RQs, there Listed Species LOC would still be exceeded (**Table 5.12**). The most sensitive amphibian toxicity values for acid/ salt (Western chorus frog tadpoles LC_{50} = 181 mg a.e./L, E61180) and ester (Leopard frog tadpoles LC_{50} = 0.505 mg a.e./L, MRID 445173-05) are used to calculate RQs. The LOCs for ester direct application to water scenarios would still be exceeded with RQs ranging from 1.5 to 7.9; however, the LOC is no longer exceeded for acid/salt direct application to water scenarios, rice applications (RQ = 0.008), or for the Forestry/Tree and Brush Control (ester drift+runoff, aerial) scenario (RQ = 0.04).

Table 5.12 Acute RQs for amphibians and EECs for direct application to water to represent 2,4-D acid, salt, and ester uses (based on the most sensitive amphibian toxicity data)

Master Label Use Category	Model Scenario	Method[1]	Application Rate	Peak EEC (µg/L)	Acute RQ*	
					Acid/salt	Ester
Aquatic Weed Control (surface application or subsurface injection)	Direct water applications	G & A	10.8 lb a.e./acre-ft (to achieve 4 ppm concentration)	4000[2]	0.02	**7.9***
Aquatic Weed Control	Direct water applications	G & A	2 app @2 lb a.e./acre (30-day interval)	740	0.004	**1.5***
Aquatic Weed Control	Direct water applications	G & A	2 app @ 4 lb a.e./ acre (21-day interval)	1480	0.008	**2.9***

*LOC exceedances (acute RQ ≥ 0.05 are bolded; Acute RQ (acid and salts) = use-specific peak EEC / 181 mg a.e./L (E61180 western chorus frog tadpole). Acute RQ (esters) = use-specific peak EEC / 0.505 mg a.e./L (MRID 445173-05 leopard frog tadpole).
[1]G = ground application. A = aerial application.
[2]Aquatic weed control-peak water concentration: 4000 µg/L , For ester direct application scenarios, 2,4-D acid input parameters were used to determine EEC. All other runoff and drift application scenarios used 2,4-D ester input parameters to determine the EEC.

5.2.1.2 Terrestrial-Phase CRLF and AW

The terrestrial-phase of the CRLF considers juvenile and adult life stages during which much time is spent in a terrestrial habitat. Submerged terrestrial-phase CRLFs are not considered here; their exposure is addressed as an aquatic-phase CRLF. Life history for the AW states that it is an obligatory terrestrial organism. Since no toxicity data were available for terrestrial-phase amphibians or reptiles, toxicity data for birds were used a surrogate.

LOC exceedances:
All the 2,4-D modeled uses except Citrus and Potatoes have the potential to directly affect the CRLF and AW based on the acute LOC exceedances demonstrated in T-REX. Based on T-REX, the only use with chronic direct effect concerns is aquatic weed control via surface application.

T-HERPS refinements:
Because the above risk estimation identified LOC exceedances for the terrestrial-phase CRLF and the AW, the T-HERPS model was used as a standard protocol for further refining the assessment for direct effects to the CRLF and the AW. T-HERPS was used to refine acute dose-based, chronic-dietary, and sub-acute dietary risks to the terrestrial-phase CRLF and AW via consumption of large insects, small herbivorous mammals, small insectivorous mammals, and small terrestrial-phase amphibians exposed to liquid applications already identified by T-REX. Dose-based acute RQs exceeding Listed Species LOCs ranged from 0.28-2.01(**Tables 5.13.a to 5.13.c**), and dietary-based chronic RQs exceeding Chronic LOC ranged from 7.58-8.88 (**Table 5.13.d**). Based on the results of the T-HERPS model, all the modeled uses except uses on potato and citrus resulted in LOC exceedances, although there were fewer exceedances for smaller organisms (14 g) than there were for larger organisms (238 g).

Table 5.13.a Summary of T-HERPS Terrestrial-Phase Amphibian Dose-based RQ Exceedances for Direct Effects to the (small, 14 g) CRLF and AW from Ingestion of Residues on or in Prey Items (Based on Liquid Applications of 2,4-D)					
Modeling Scenario	**Method[1]**	**Application Rate**	**Food Item**	**Small (14 g)**	
				Dose-based EEC (ppm)	**Dose-based Acute RQ***
Direct water application use					
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[2]	Broadleaf plants and small insects	283.23	**0.95**
			Fruits/pods/seeds and large insects	31.47	**0.11**

[1]G = ground application. A = aerial application.
[2]Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre
*All bolded values exceed Level of Concern (LOC) for the following risk categories:
Acute Risk to Herpetofauna Dietary items 0.5
Herpetofauna Dietary items for Acute Restricted Use 0.2
Acute Listed Species of Herpetofauna Dietary items 0.1

Table 5.13.b Summary of T-HERPS Terrestrial-Phase Amphibian Dose-based RQ Exceedances for Direct Effects to the (medium, 37 g) CRLF and AW from Ingestion of Residues on or in Prey Items (Based on Liquid Applications of 2,4-D)					
Modeling Scenario	**Method[1]**	**Application Rate**	**Food Item**	**Medium (37 g)**	
				Dose-based EEC (ppm)	**Dose-based Acute RQ***
Orchard Uses					
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	327.36	**1.10**
Filberts	G	4 apps @ 0.5 lb a.e./acre (30-day interval)	Small herbivore mammals	82.57	**0.28**
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	Small herbivore mammals	203.45	**0.68**
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	Small herbivore mammals	229.15	**0.77**
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	Small herbivore mammals	300.01	**1.01**
Agricultural – Food Crop Uses					

Table 5.13.b Summary of T-HERPS Terrestrial-Phase Amphibian Dose-based RQ Exceedances for Direct Effects to the (medium, 37 g) CRLF and AW from Ingestion of Residues on or in Prey Items (Based on Liquid Applications of 2,4-D)					
Modeling Scenario	**Method[1]**	**Application Rate**	**Food Item**	**Medium (37 g)**	
				Dose-based EEC (ppm)	**Dose-based Acute RQ***
Field Corn, Popcorn	A/G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	Small herbivore mammals	230.88	**0.77**
Sweet Corn	A/G	1 app @ 1 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29	Small herbivore mammals	149.60	**0.50**
Sugarcane	A/G	2 apps @ 2 lb a.e./acre (20-day interval)	Small herbivore mammals	361.11	**1.21**
Cereal Grains	A/G	1 post-emergence app @ 1.25 lb a.e./acre, 1 pre-harvest app @ 0.5 lb a.e./acre (90-day interval)	Small herbivore mammals	187.00	**0.63**
Grain or Forage Sorghum	A/G	1 post-emergence app @ 1.0 lb a.e./acre	Small herbivore mammals	149.60	**0.50**
Hops	A/G	3 apps @ 0.5 lb a.e./acre (30-day interval)	Small herbivore mammals	82.50	**0.28**
Asparagus	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	327.36	**1.10**
Fallowland and Crop Stubble	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	327.36	**1.10**
Agricultural – Non-food Crop Uses					
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	327.36	**1.10**
Non-agricultural Uses					
Non-cropland	A/G	1 app @ 4 lb a.e./acre	Small herbivore mammals	598.39	**2.01**
			Small insectivore mammals	37.40	**0.13**
Forestry	A/G	1 app @ 4 lb a.e./acre	Small herbivore mammals	598.39	**2.01**
			Small insectivore mammals	37.40	**0.13**
Tree and Brush Control	A/G	1 app @ 4 lb a.e./acre	Small herbivore mammals	598.39	**2.01**
			Small insectivore	37.40	**0.13**

Table 5.13.b Summary of T-HERPS Terrestrial-Phase Amphibian Dose-based RQ Exceedances for Direct Effects to the (medium, 37 g) CRLF and AW from Ingestion of Residues on or in Prey Items (Based on Liquid Applications of 2,4-D)					
Modeling Scenario	**Method[1]**	**Application Rate**	**Food Item**	**Medium (37 g)**	
				Dose-based EEC (ppm)	**Dose-based Acute RQ***
			mammals		
Ornamental Turf	A/G	2 apps @ 1.5 lb a.e./acre (21-day interval)	Small herbivore mammals	267.32	**0.90**
Grass Grown for Seed and Sod	A/G	2 apps @ 2 lb a.e./acre (21-day interval)	Small herbivore mammals	356.42	**1.20**
Direct Application to Water Uses					
Rice	A/G	1 app @ 1.5 lb a.e./acre	Small herbivore mammals	224.40	**0.75**
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[2]	Small herbivore mammals	8078.29	**27.11**
			Small insectivore mammals	504.89	**1.69**
			Broadleaf plants and small insects	278.35	**0.93**
			Fruits/pods /seeds and large insects	30.93	**0.10**
Aquatic Weed Control	A/G	2 app @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	327.36	**1.10**
Aquatic Weed Control	A/G	2 app @ 4 lb a.e./acre (21-day interval)	Small herbivore mammals	712.84	**2.39**
			Small insectivore mammals	44.55	**0.15**

[1]G = ground application. A = aerial application.
[2] Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre
*All bolded values exceed Level of Concern (LOC) for the following risk categories:
Acute Risk to Herpetofauna Dietary items 0.5
Herpetofauna Dietary items for Acute Restricted Use 0.2
Acute Listed Species of Herpetofauna Dietary items 0.1

Table 5.13.c Summary of T-HERPS Terrestrial-Phase Amphibian Dose-based RQ Exceedances for Direct Effects to the (large, 238 g) CRLF and AW from Ingestion of Residues on or in Prey Items (Based on Liquid Applications of 2,4-D)					
Modeling Scenario	**Method[1]**	**Application Rate**	**Food Item**	**Large (238 g)**	
				Dose-based EEC (ppm)	**Dose-based Acute RQ***
Orchard Uses					
Nut Orchards, Pistachios	G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	50.89	**0.17**
Grapes (all)	G	1 app @ 1.36 lb a.e./acre	Small herbivore mammals	31.63	**0.11**
Blueberries	G	2 apps @ 1.4 lb a.e./acre (30-day interval)	Small herbivore mammals	35.62	**0.12**
Stone and Pome Fruits	G	2 apps @ 2 lb a.e./acre (75-day interval)	Small herbivore mammals	48.64	**0.16**
Agricultural – Food Crop Uses					
Field Corn, Popcorn	A/G	1 app @ 1.0 lb a.e./acre March 15, 1 app @ 0.5 lb a.e./acre April 29, 1 app @ 1.5 lb a.e./acre August 15	Small herbivore mammals	35.89	**0.12**
Sugarcane	A/G	2 apps @ 2 lb a.e./acre (20-day interval)	Small herbivore mammals	56.14	**0.19**
Asparagus	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	50.89	**0.17**
Fallowland and Crop Stubble	A/G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	50.89	**0.17**
Agricultural – Non-food Crop Uses					
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	G	2 apps @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	50.89	**0.17**
Non-agricultural Uses					
Non-cropland	A/G	1 app @ 4 lb a.e./acre	Small herbivore mammals	93.03	**0.31**
Forestry	A/G	1 app @ 4 lb a.e./acre	Small herbivore mammals	93.03	**0.31**
Tree and Brush Control	A/G	1 app @ 4 lb a.e./acre	Small herbivore mammals	93.03	**0.31**
Ornamental Turf	A/G	2 apps @ 1.5 lb a.e./acre (21-day interval)	Small herbivore mammals	41.56	**0.14**

Table 5.13.c Summary of T-HERPS Terrestrial-Phase Amphibian Dose-based RQ Exceedances for Direct Effects to the (large, 238 g) CRLF and AW from Ingestion of Residues on or in Prey Items (Based on Liquid Applications of 2,4-D)					
Modeling Scenario	**Method[1]**	**Application Rate**	**Food Item**	**Large (238 g)**	
				Dose-based EEC (ppm)	**Dose-based Acute RQ***
Grass Grown for Seed and Sod	A/G	2 apps @ 2 lb a.e./acre (21-day interval)	Small herbivore mammals	55.41	**0.19**
Direct Application to Water Uses					
Rice	A/G	1 app @ 1.5 lb a.e./acre	Small herbivore mammals	34.89	**0.12**
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[2]	Small herbivore mammals	1255.87	**4.21**
			Small insectivore mammals	78.49	**0.26**
			Broadleaf plants and small insects	182.43	**0.61**
Aquatic Weed Control	A/G	2 app @ 2 lb a.e./acre (30-day interval)	Small herbivore mammals	50.89	**0.17**
Aquatic Weed Control	A/G	2 app @ 4 lb a.e./acre (21-day interval	Small herbivore mammals	110.82	**0.37**

[1]G = ground application. A = aerial application.
[2] Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre.
*All bolded values exceed Level of Concern (LOC) for the following risk categories:
Acute Risk to Herpetofauna Dietary items 0.5
Herpetofauna Dietary items for Acute Restricted Use 0.2
Acute Listed Species of Herpetofauna Dietary items 0.1

Table 5.13.d Summary of T-HERPS Terrestrial-Phase Amphibian Dietary-based RQ Exceedances for Direct Effects to the CRLF and AW from Ingestion of Residues on or in Prey Items (Based on Liquid Applications of 2,4-D)					
Modeling Scenario	**Method[1]**	**Application Rate**	**Food Item**	**Dietary-based EEC (ppm)**	**Dietary-based Chronic RQ***
Direct Application to Water Uses					
Aquatic Weed Control	A/G	1 app @ 10.8 lb a.e./acre foot[2]	Small herbivore mammals	8539.90	**8.88**
			Broadleaf plants and small insects	7290.00	**7.58**
[1]G = ground application. A = aerial application. [2]Label states apply 10.8 lb a.e./acre-foot. If water body is 5 ft deep, this equals an application rate of 54 lb a.e/.acre. *All bolded values exceed Level of Concern (LOC) for the following risk categories: Chronic Risk to Herpetofauna Dietary items 1					

5.2.2 Indirect Effects (via Reductions in Prey Base)

5.2.2.1 Algae (Non-vascular Plants)

As discussed in **Section 2.5.3**, the diet of CRLF tadpoles is composed primarily of unicellular aquatic plants (*i.e.*, algae and diatoms) and detritus.

LOC exceedances:
LOCs were exceeded for the direct application to water aquatic weed control scenarios. For acid/salt aquatic weed control uses, the RQ was 1.03. For ester aquatic weed control uses, the RQ was 60.61.

Comparison of modeled to observed water concentrations:
The available monitoring data have been presented in **Section 3.2.7**. Compared with the modeling results, the modeled values are much higher than USGS NAWQA and CDPR data for California. The lack of agreement between the model and monitoring results is not unexpected since the monitoring data were not designed to target areas of 2,4-D usage. With the exception of direct aquatic applications, such as rice use and aquatic weed control, the model predictions are comparable with the available registrant-submitted fate studies.

5.2.2.2 Aquatic Invertebrates

The potential for 2,4-D to elicit indirect effects to the CRLF via effects on freshwater invertebrate food items is dependent on several factors including: (1) the potential magnitude of effect on freshwater invertebrate individuals and populations; and (2) the number of prey species potentially affected relative to the expected number of species needed to maintain the dietary needs of the CRLF. Together, these data provide a basis to evaluate whether the number of individuals within a prey species is likely to be reduced such that it may indirectly affect the CRLF.

LOC exceedances:
Acute LOC exceedances were observed in the direct application to water scenarios. For rice, the RQ was 0.06. For the acid/salt aquatic weed control use, the RQ was 0.16. For the ester aquatic weed control use, the RQ was 1.82. There were no chronic exceedances.

Comparison of modeled to observed water concentrations:
The available monitoring data have been presented in **Section 3.2.7**. Compared with the modeling results, the modeled values are much higher than USGS NAWQA and CDPR data for California. The lack of agreement between the model and monitoring results is not unexpected since the monitoring data were not designed to target areas of 2,4-D usage. With the exception of direct aquatic applications, such as rice use and aquatic weed control, the model predictions are comparable with the available registrant-submitted fate studies.

5.2.2.3 Fish and Aquatic-phase Frogs

Evidence for indirect effects to fish and frogs as food items is the same as presented the direct effects analysis for aquatic-phase CRLFs (**Section 5.2.1.1**).

5.2.2.4 Mammals

Life history data for terrestrial-phase CRLFs and AW indicate that large adult frogs and AW consume small mammals.

LOC exceedances:
Acute dose-based RQs for all liquid and granular applications of 2,4-D except Citrus and Potato exceeded the LOCs for small mammalian prey items.

Chronic RQ values representing 2,4-D exposures to small mammals indicate risks resulting from some application scenarios. Dose-based chronic RQs exceeded the LOC for all liquid applications. Dietary-based chronic RQ exceeded the LOC for liquid applications of 4 applications @ 0.5 lb a.e./acre and greater.

Percent effect analysis:
A percent effect analysis was conducted by determining an expected percent effect on the prey item (small mammals) at the RQ, implying effect at the calculated EEC. The percent effect ranged from 0.003% to 100% depending on cropping scenario (**Table 5.14**).

Citrus and Potatoes

Although the mammalian dose-based chronic LOC was exceeded for both the CRLF and the AW prey, EFED determined that this effect would be insignificant as the potential small effect on mammal reproduction (as prey of the CRLF and AW) would not likely impact the overall prey base. It is anticipated that any effects would be small since the RQs only mildly exceeded the LOC.

5.2.2.5 Birds (assessed for AW only)

Life history data for the AW indicates that AWs consume small birds.

LOC exceedances:

RQ values, estimated using T-REX, representing direct exposures of 2,4-D to the AW are used to represent risks of 2,4-D to small birds in terrestrial habitats. The indirect effects to birds as food items are based on the direct effects analysis for the AW (**Section 5.2.1.2**).

Percent effect analysis:

A percent effect analysis was conducted by determining an expected percent effect on the prey item (small birds) at the RQ, implying effect at the calculated EEC. The percent effect ranged from 0.003% to 100% depending on cropping scenario (**Table 5.14**).

Citrus and Potatoes

Although the avian acute dose-based LOC was exceeded for AW prey, EFED determined that this effect was discountable and insignificant as the predicted percentage of acute effect was only 0.0033% of the bird population (birds as prey items of the AW), and if even if this effect did occur, the overall prey base of the AW would likely not be affected.

Table 5.14 Summary of Indirect Effect RQs for the Terrestrial–phase CRLF and AW, Percent Effect Probabilities

Master Label Use Category	Indirect effects to CRLF and AW		Indirect effects to AW	
	Highest – Dose-based RQ for Mammals [1]	Percent Effect for Mammals [2]	Highest Dose-based RQ for Birds [1]	Percent Effect for Birds [2]
Orchard Uses				
Nut Orchards, Pistachios	0.52	10%	2.79	98%
Filberts	0.13	0.003%	0.70	24%
Grapes (all)	0.32	1%	1.73	86%
Blueberries	0.36	2%	1.95	90%
Stone and Pome Fruits	0.47	7%	2.55	97%
Citrus	No LOC exceedance	N/A	0.13	0.0033%
Agricultural – Food Crop Uses				
Field Corn, Popcorn	0.72(G)	26%	2.43(G)	96%
Sweet Corn	0.72(G)	26%	2.43(G)	96%

Table 5.14 Summary of Indirect Effect RQs for the Terrestrial–phase CRLF and AW, Percent Effect Probabilities				
Master Label Use Category	**Indirect effects to CRLF and AW**		**Indirect effects to AW**	
	Highest – Dose-based RQ for Mammals [1]	**Percent Effect for Mammals [2]**	**Highest Dose-based RQ for Birds [1]**	**Percent Effect for Birds [2]**
Potatoes	No LOC exceedance	N/A	0.13	0.0033%
Sugarcane	0.57	14%	3.07	99%
Cereal Grains	0.30	0.9%	1.59	82%
Grain or Forage Sorghum	0.72(G)	26%	2.43(G)	96%
Hops	0.13	0.003%	0.70	24%
Asparagus	0.52	10%	2.79	98%
Fallowland and Crop Stubble	0.52	10%	2.79	98%
Agricultural – Non-food Crop Uses				
Established Grass Pastures, Rangeland, Perennial Grassland Not in Agricultural Production	0.52	10%	2.79	98%
Non-agricultural Uses				
Non-cropland	2.86 (G)	98%	9.70 (G)	100%
Forestry	0.94	45%	5.09	100%
Tree and Brush Control	0.94	45%	5.09	100%
Ornamental Turf	1.07G)	55%	3.64(G)	99%
Grass Grown for Seed and Sod	1.43 (G)	76%	4.85 (G)	100%
Direct Application to Water Uses				
Aquatic Weed Control Ditchbank	1.43 (G)	76%	4.85 (G)	100%
Aquatic Weed Control Surface application	2.86 (G)	98%	9.70 (G)	100%
Aquatic Weed Control Surface application or subsurface injection	38.68 (G)	100%	130.96 (G)	100%

[1]G = granular application. All other applications are liquid.
[2]A slope value was not available for the acute bird of LD_{50} 298 mg a.e./kg-bw (MRID 442757-0) and mammals of LD_{50} 441 mg a.e./kg –bw (MRID 414135-01), therefore the probability was calculated based on the default slope value of 4.5. Confidence intervals (2,9)

5.2.2.6 Terrestrial Invertebrates

When the terrestrial-phase CRLF reaches juvenile and adult stages, its diet is mainly composed of terrestrial invertebrates. Life history data for the AW state that the diet of the AW also includes invertebrates and may depend on an individual's size, sex, age, and location. As previously discussed in **Section 5.1.2.1**, indirect effects to the terrestrial-phase CRLF and AW via reduction in terrestrial invertebrate prey items that are exposed to liquid and granular applications of 2,4-D are expected.

LOC exceedances:
RQs for all liquid and granular applications of 2,4-D except citrus and potato uses exceeded the LOCs for both small and large invertebrate prey items, as well as for earthworms.

Earthworm risks:
Risks to terrestrial invertebrates can also be estimated using the available earthworm toxicity data. The LC_{50} for earthworms was 61.6 µg a.e./cm^2 which is equivalent to 5.50 lb a.e./acre. RQs were calculated as a ratio of the application rate and the toxicity value (**Table 5.15**). Based on these analyses, 2,4-D has the potential to indirectly affect those listed species that rely on terrestrial invertebrates during at least some portion of their life-cycle (*i.e.*, CRLF and AW).

Table 5.15 Acute RQs used to Estimate Indirect effects to terrestrial-phase CRLFs and AW via ingestion of terrestrial invertebrates (represented by earthworms)

EEC (lb a.e./acre) [1]	Acute RQ[3]
0.07	0.01
0.10	0.02
0.50	**0.10***
1.0	**0.19***
1.36	**0.25***
1.4	**0.27***
1.5	**0.29***
2.0	**0.39***
4.0	**0.78***

[1]Single application rates from a variety of crops are represented here.
**Acute RQ ≥ 0.05 exceeds acute level of concern (LOC) for terrestrial invertebrates.

5.2.2.7 Frogs

Terrestrial-phase adult CRLFs and AW also consume small frogs. RQ values, estimated using T-REX, representing direct exposures of 2,4-D to terrestrial-phase CRLFs and AW are used to represent exposures of 2,4-D to small frogs in terrestrial habitats. The indirect effects to frogs as food items are based on the direct effects analysis for the terrestrial-phase CRLF and AW (**Section 5.2.1.2**).

5.2.3 Indirect Effects (via Habitat Effects)

5.2.3.1 Aquatic Plants (Vascular and Non-vascular)

Aquatic plants serve several important functions in aquatic ecosystems. Non-vascular aquatic plants are primary producers and provide the autochthonous energy base for aquatic ecosystems. Vascular plants provide structure as attachment sites and refugia for many aquatic invertebrates, fish, and juvenile organisms, such as fish and frogs. In addition, vascular plants also provide primary productivity and oxygen to the aquatic ecosystem. Rooted plants help reduce sediment loading and provide stability to near-shore areas and lower streambanks. In addition, vascular aquatic plants are important as attachment sites for egg masses of CRLFs. Potential indirect effects to the CRLF based on impacts to habitat and/or primary production were assessed using RQs from freshwater aquatic vascular and non-vascular plant data.

LOC exceedances:
For non-vascular plants, LOCs were exceeded for the direct application to water aquatic weed control scenarios. For acid/salt aquatic weed control uses, the RQ was 1.03. For ester aquatic weed control uses, the RQ was 60.61.

For vascular plants, there were several LOC exceedances for acid/salt uses for vascular aquatic plants (see **Table 5.4.a**). There were no LOC exceedances for drift+runoff ester uses and drift only ester uses that were not direct application to water. The LOC was exceeded for the rice and all direct application to water uses for both acid/salts and esters.

Comparison of modeled to observed water concentrations:
The available monitoring data have been presented in **Section 3.2.7**. Compared with the modeling results, the modeled values are much higher than USGS NAWQA and CDPR data for California. The lack of agreement between the model and monitoring results is not unexpected since the monitoring data were not designed to target areas of 2,4-D usage. With the exception of direct aquatic applications, such as rice use and aquatic weed control, the model predictions are comparable with the available registrant-submitted fate studies.

5.2.3.2 Terrestrial Plants

Terrestrial plants serve several important habitat-related functions for the CRLF and the AW. In addition to providing habitat and cover for invertebrate and vertebrate prey items of the CRLF and the AW, terrestrial vegetation also provides shelter for the CRLF and the AW and cover from predators while foraging. Terrestrial plants also provide energy to the terrestrial ecosystem through primary production. Upland vegetation including grassland and woodlands provides cover during dispersal. Riparian vegetation helps to maintain the integrity of aquatic systems by providing bank and thermal stability, serving as a buffer to filter out sediment, nutrients, and contaminants before they reach the watershed, and serving as an energy source.

Loss, destruction, and alteration of habitat were identified as threats to the CRLF in the USFWS Recovery Plan (USFWS, 2002). Herbicides can adversely impact habitat in a number of ways. In the most extreme case, herbicides in spray drift and runoff from the site of application have the potential to kill (or reduce growth and/or biomass) all or a substantial amount of the vegetation, thus removing or impacting structures that define the habitat, and reducing the functions (*e.g.*, cover, food supply for prey base) provided by the vegetation.

Riparian vegetation typically consists of three tiers of vegetation, which include a groundcover of grasses and forbs, an understory of shrubs and young trees, and an overstory of mature trees. Frogs spend a considerable amount of time resting and feeding in riparian vegetation; the moisture and cover of the riparian plant community provides good foraging habitat and may facilitate dispersal in addition to providing pools and backwater aquatic areas for breeding (USFWS, 2002). According to Hayes and Jennings (1988), the CRLF tends to occupy water bodies with dense riparian vegetation including willows (*Salix* sp.). Upland habitat includes grassland and woodlands, as well as scrub/shrub habitat.

All of the modeled uses of 2,4-D exceed the Agency LOCs for risk for terrestrial plants including both monocots and dicots. In addition, there are a multitude of reported incidents of 2,4-D negatively impacting terrestrial plants (**Section 4.4.2).**

Although the terrestrial plant LOC was exceeded for Citrus and Potatoes for both CRLF and the AW, EFED determined the effect to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.

Based on exceedances of the terrestrial plant LOCs for all 2,4-D use patterns following runoff and spray drift to dry and semi-aquatic areas, the following general conclusions can be made with respect to potential harm to riparian habitat:

- 2,4-D may enter riparian areas via runoff and/or spray drift where it may contact foliar surfaces of emerged seedlings or form a chemical barrier on soil, which would affect pre-emergent plants.

- Based on 2,4-D's mode of action and a comparison of seedling emergence and vegetative vigor EC_{25} values to EECs estimated using TerrPlant, emerging or developing seedlings may be affected in areas receiving both runoff and drift and in areas receiving drift alone at applications rates greater than a single application of 0.07 lb a.e./acre. If inhibition of new growth occurs, it could result in degradation of high quality riparian habitat over time because as older growth dies from natural or anthropogenic causes, plant biomass may be prevented from being replenished in the riparian area.

In summary, terrestrial plant RQs exceed LOCs, which indicates risk to upland and riparian vegetation. However, while it is not expected that woody plants with mature bark are sensitive to environmentally relevant 2,4-D concentrations, the lack of a guideline study on established woody plants precludes estimation of effects. Because upland and riparian areas are comprised of a mixture of both woody plants and herbaceous vegetation, terrestrial-phase CRLFs and the AW may be indirectly affected by adverse effects solely to herbaceous vegetation, which provides habitat and cover for the CRLF, AW and their prey.

5.2.3.2.1 Spray Drift Buffer Analysis

In order to estimate buffer distances that are protective of plant species that the terrestrial-phase CRLF and AW or their prey may depend on for food and cover, AgDRIFT was used to model the dissipation distance to the EC_{25} levels for terrestrial plants. Input parameters for AgDRIFT for aerial and ground applications are described in **Table 5.16.** For ground applications, only Tier I model estimates are available; the maximum buffer distance that can be calculated in 1000 ft. For aerial applications, Tier I and Tier II models provide estimates of 1000 feet or less; the Tier III model provides estimates of up to 2640 ft.

Because 2,4-D is used as a pre-emergent and post-emergent herbicide, buffer distances were calculated for the most sensitive endpoints for both monocots and dicots in the seedling emergence and vegetative vigor studies. For ground application effects on monocots, required buffer distances to eliminate LOC exceedances ranged from 0 to 115 ft. For ground application effects on dicots, required buffer distances to eliminate LOC exceedances ranged from 16 to >1000 ft. For aerial application effects on monocots, required buffer distances to eliminate LOC exceedances ranged from 0 to 2402 ft. For aerial application effects on dicots, required buffer distances to eliminate LOC exceedances ranged from 154 to >2640 ft.

This analysis did not include any mitigation resulting from the RED regarding spray drift management requirements. If these conditions were incorporated into the analysis, it it likely that the estimated buffer widths in **Table 5.16** would be reduced.

Table 5.16 Estimation of Buffer Distance Required to Eliminate LOC Exceedances (only spray drift exposure considered) for Terrestrial Plants Based on AgDRIFT				
	EC_{25} (lb a.e./ac)	Fraction of applied = EC_{25} ÷ Rate	Buffer Width, aerial (ft) [1]	Buffer Width, ground (ft) [2]
Application Rate = 0.07 lb a.e./ac				
Seedling Emergence Monocots	0.097	1.386	0 (TI)	0
Seedling Emergence Dicots	0.012	0.171	154.2 (TI)	16.4
Vegetative Vigor Monocots	0.088	1.257	0 (TI)	0
Vegetative Vigor Dicots	0.0021	0.03	1807.72 (TIII)	85.3
Application Rate = 0.1 lb a.e./ac				
Seedling Emergence Monocots	0.097	0.97	0 (TI)	3.28
Seedling Emergence Dicots	0.012	0.12	242.78 (TI)	22.97
Vegetative Vigor Monocots	0.088	0.88	0 (TI)	3.28
Vegetative Vigor Dicots	0.0021	0.021	2480.29 (TIII)	118.11
Application Rate = 0.5 lb a.e./ac				
Seedling Emergence Monocots	0.097	0.194	131.23 (TI)	16.4
Seedling Emergence Dicots	0.012	0.024	2250.63 (TIII)	104.99
Vegetative Vigor Monocots	0.088	0.176	150.92 (TI)	16.4
Vegetative Vigor Dicots	0.0021	0.0042	>2640 (TIII)	475.72
Application Rate = 1 lb a.e./ac				
Seedling Emergence Monocots	0.097	0.097	318.24 (TI)	29.53
Seedling Emergence Dicots	0.012	0.012	>2640 (TIII)	200.13
Vegetative Vigor Monocots	0.088	0.088	357.61 (TI)	29.53
Vegetative Vigor Dicots	0.0021	0.0021	>2640 (TIII)	770.99
Application Rate = 1.36 lb a.e./ac				
Seedling Emergence Monocots	0.097	0.0713	465.87 (TI)	36.09
Seedling Emergence Dicots	0.012	0.0088	>2640 (TIII)	265.74
Vegetative Vigor Monocots	0.088	0.0647	524.93 (TI)	39.37
Vegetative Vigor Dicots	0.0021	0.00154	>2640 (TIII)	944.87
Application Rate = 1.4 lb a.e./ac				

Table 5.16 Estimation of Buffer Distance Required to Eliminate LOC Exceedances (only spray drift exposure considered) for Terrestrial Plants Based on AgDRIFT				
	EC_{25} (lb a.e./ac)	**Fraction of applied = EC_{25} ÷ Rate**	**Buffer Width, aerial (ft)[1]**	**Buffer Width, ground (ft)[2]**
Seedling Emergence Monocots	0.097	0.0693	482.28 (TI)	39.37
Seedling Emergence Dicots	0.012	0.00857	>2640 (TIII)	269.03
Vegetative Vigor Monocots	0.088	0.062857	547.89 (TI)	42.65
Vegetative Vigor Dicots	0.0021	0.0015	>2640 (TIII)	961.27
Application Rate = 1.5 lb a.e./ac				
Seedling Emergence Monocots	0.097	0.06467	528.21 (TI)	39.37
Seedling Emergence Dicots	0.012	0.008	>2640 (TIII)	285.43
Vegetative Vigor Monocots	0.088	0.05867	600.39 (TI)	45.93
Vegetative Vigor Dicots	0.0021	0.0014	>2640 (TIII)	>1000
Application Rate = 2.0 lb a.e./ac				
Seedling Emergence Monocots	0.097	0.0485	777.55 (TI)	52.49
Seedling Emergence Dicots	0.012	0.006	>2640 (TIII)	360.89
Vegetative Vigor Monocots	0.088	0.044	905.5 (TI)	59.05
Vegetative Vigor Dicots	0.0021	0.00105	>2640 (TIII)	>1000
Application Rate = 4.0 lb a.e./ac				
Seedling Emergence Monocots	0.097	0.02425	2230.94 (TIII)	104.99
Seedling Emergence Dicots	0.012	0.003	>2640 (TIII)	606.95
Vegetative Vigor Monocots	0.088	0.022	2401.55 (TIII)	114.83
Vegetative Vigor Dicots	0.0021	0.000525	>2640 (TIII)	>1000

[1]Aerial application scenarios are modeled with AgDrift Tier I (TI) and AgDrift Tier III (TIII).
[2]Ground application scenarios are modeled with AgDrift Tier I, no higher tiers available.

5.2.4 Modification to Designated Critical Habitat

5.2.4.1 Aquatic-phase PCEs

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the CRLF are related to potential effects to aquatic and/or terrestrial plants:

- Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.
- Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae).

Conclusions for potential indirect effects to the CRLF via direct effects to aquatic and terrestrial plants are used to determine whether modification to critical habitat may occur. There is a potential for habitat modification via impacts to aquatic plants (**Sections 5.2.2.1** and **5.2.3.1**) and terrestrial plants (**Section 5.2.3.2**)

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." Other than impacts to algae as food items for tadpoles (discussed above), this PCE is assessed by considering direct and indirect effects to the aquatic-phase CRLF via acute and chronic freshwater fish and invertebrate toxicity endpoints as measures of effects. There is a potential for habitat modification via impacts to aquatic-phase CRLFs (**Section 5.2.1.1**) and effects to freshwater invertebrates and fish as food items (**Sections 5.2.2.2** and **5.2.2.3**).

5.2.4.2 Terrestrial-Phase PCEs

Two of the four assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the CRLF and AW are related to potential effects to terrestrial plants:

- Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs and AWs: Upland areas within 200 ft of the edge of the riparian vegetation or drip line surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provide the CRLF and AW shelter, forage, and predator avoidance.

- Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal.

As discussed above, there is potential for habitat modification of the terrestrial-phase CRLF and AW via impacts to terrestrial plants as indicated by potential impacts to herbaceous vegetation, which provides habitat, cover, and a means of dispersal for the terrestrial-phase CRLF and AW and their prey. This habitat modification could be caused by all modeled uses of 2,4-D at the maximum labeled rate.

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of 2,4-D on this PCE, acute toxicity endpoints for terrestrial invertebrates and acute and chronic toxicity endpoints for mammals and terrestrial-phase frogs are used as measures of effects. Based on the characterization of indirect effects to the terrestrial-phase CRLF and the AW via reduction in prey base (**Section 5.2.2.4** for terrestrial invertebrates, **Section 5.2.2.5** for mammals, and **Section 5.2.2.6** for frogs), there is potential for critical habitat modification via a reduction of terrestrial invertebrates, small mammals, and frogs as food items.

The fourth terrestrial-phase PCE is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs, as well as the AW, and their food sources. As discussed in **Section 5.2.1.2**, direct acute effects to the terrestrial-phase CRLF and the AW are likely. Indirect effects to the terrestrial-phase CRLF and AW via reduction in prey base are likely. Therefore, there is potential for habitat modification via direct and indirect effects to the terrestrial-phase CRLF and AW.

6. Uncertainties

6.1 Exposure Assessment Uncertainties

6.1.1 Maximum Use Scenario

The screening-level risk assessment focuses on characterizing potential ecological risks resulting from a maximum use scenario, which is determined from labeled statements of maximum application rate and number of applications with the shortest time interval between applications. The frequency at which actual uses approach this maximum use scenario may be dependent on pest resistance, timing of applications, cultural practices, and market forces.

6.1.2 Usage Uncertainties

County-level usage data were obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Eight years of data (1999 – 2006) were included in this analysis. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. In addition, it is possible that the data may contain reports for pesticide uses that have been cancelled. The CDPR PUR data does not include homeowner-applied pesticides; therefore, residential uses are not likely to be reported. As with all pesticide usage data, there may be instances of misuse and misreporting. The Agency made use of the most current, verifiable information; in cases where there were discrepancies, the most conservative information was used.

6.1.3 Aquatic Exposure Modeling of 2,4-D

Aquatic exposures are quantitatively estimated for all of the assessed uses using scenarios that represent high exposure sites for 2,4-D application. The standard ecological water body scenario (EXAMS pond) used to calculate potential aquatic exposure to pesticides is intended to represent conservative estimates and to avoid underestimations of the actual exposure. Each of these sites represents a 10-hectare field that drains into a 1-hectare pond, which is 2 meters deep and has no outlet. Exposure estimates generated using the standard pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and first-order streams. As a group, there are factors that make these water bodies more or less vulnerable than the standard surrogate pond. Static water bodies that have larger ratios of drainage area to water body volume would be expected to have higher peak EECs than the standard pond. These water bodies will be either shallower or have large drainage areas (or both). Shallow water bodies tend to have limited additional storage capacity and, thus, tend to overflow and carry pesticide in the discharge whereas the standard pond has no discharge. As watershed size increases beyond 10 hectares, at some point, it becomes unlikely that the entire watershed is planted to a single crop, which is all treated with the pesticide. Headwater streams can also have peak concentrations higher than the standard pond, but they tend to persist for only short periods of time and are then carried downstream.

6.1.3.1 PRZM/EXAMS

The Agency acknowledges that there are some unique aquatic habitats that are not accurately captured by this modeling scenario and modeling results may, therefore, under- or over-estimate exposure, depending on a number of variables. For example, some organisms may inhabit water bodies of different size and depth and/or are located adjacent to larger or smaller drainage areas than the EXAMS pond. In addition, the Services agree that the existing EXAMS pond represents the best currently available approach for estimating aquatic exposure to pesticides (USFWS/NMFS, 2004).

In general, the linked PRZM/EXAMS model produces estimated aquatic concentrations that are expected to be exceeded once within a ten-year period. The Pesticide Root Zone Model is a process or "simulation" model that calculates what happens to a pesticide in an agricultural field on a day-to-day basis. It considers factors such as rainfall and plant transpiration of water, as well as how and when the pesticide is applied. It has two major components: hydrology and chemical transport. Water movement is simulated by the use of generalized soil parameters, which include field capacity, wilting point, and saturation water content. The chemical transport component can simulate pesticide application on the soil or on the plant foliage. Dissolved, adsorbed, and vapor-phase concentrations in the soil are estimated by simultaneously considering the processes of pesticide uptake by plants, surface runoff, erosion, decay, volatilization, foliar wash-off, advection, dispersion, and retardation.

Uncertainties associated with each of these individual components add to the overall uncertainty of the modeled concentrations. Additionally, model inputs from the environmental fate degradation studies are chosen to represent the upper confidence bound on the mean values that are not expected to be exceeded in the environment approximately 90 percent of the time. Mobility input values are chosen to be representative of conditions in the environment. The natural variation in soils adds to the uncertainty of modeled values. Factors such as application date, crop emergence date, and canopy cover can also affect estimated concentrations, adding to the uncertainty of modeled values. Factors within the ambient environment such as soil temperatures, sunlight intensity, antecedent soil moisture, and surface water temperatures can cause actual aquatic concentrations to differ for the modeled values.

Unlike spray drift, tools are currently not available to evaluate the effectiveness of a vegetative setback on runoff and loadings. The effectiveness of vegetative setbacks is highly dependent on the condition of the vegetative strip. For example, a well-established, healthy vegetative setback can be a very effective means of reducing runoff and erosion from agricultural fields. Alternatively, a setback of poor vegetative quality or a setback that is channelized can be ineffective at reducing loadings. Until a quantitative method to estimate the effect of vegetative setbacks on various conditions on pesticide loadings becomes available, the aquatic exposure predictions are likely to overestimate exposure where healthy vegetative setbacks exist and underestimate exposure where poorly developed, channelized, or bare setbacks exist.

In order to account for uncertainties associated with modeling, available monitoring data were compared to PRZM/EXAMS estimates of peak EECs for the different uses. As previously discussed, several data values were available from NAWQA for 2,4-D concentrations measured in surface waters receiving runoff from agricultural areas. The specific use patterns (*e.g.*, application rates and timing, crops) associated with the agricultural areas are unknown; however, they are assumed to be representative of potential 2,4-D use areas. The available monitoring data were presented in **Section 3.2.4**. Compared with the modeling results, the NAWQA and CDPR monitoring data values are lower than PRZM/EXAMS modeling results. These findings may be because the monitoring data were not designed specifically for 2,4-D use areas. Most model predictions obtained by PRZM/EXAMS are comparable with the available registrant-submitted field dissipation studies.

6.1.3.2 Direct Application to Water

Because there are no existing modeling scenarios for direct application to water, a first approximation of an EEC was predicted assuming direct application to the standard pond. For this assessment, EFED utilized a first-order decay model to estimate average concentrations that incorporates degradation based on an acceptable aerobic aquatic metabolism study ($t_{1/2}$ = 15 days, used input value of 45 days per EFED Guidance) for the EFED standard pond with no flow. This approach does not account for other types of degradation that may occur or for 2,4-D that is no longer available to aquatic plants and organisms due to sorption to sediment.

6.1.3.3 Rice Application Model

Estimates from the Tier I model generally do not represent typical concentrations found in human drinking water, as they represent paddy discharge water. However, these concentrations may be a reasonable estimate of acute concentrations for use in an ecological assessment where exposure occurs at or near the rice paddy. For both human drinking water and ecological exposure, the chronic concentrations, as well as offsite concentrations, are expected to be conservative. A higher tier rice model should be used to estimate chronic exposure to compounds that degrade rapidly into degradates that are not of risk concern.

If Tier I estimates calculated by this screening method do not exceed the level of concern in a risk assessment, there is high confidence that there will be little or no risk above the level of concern from exposure through water resources. However, when a level of concern is exceeded, it cannot be determined whether the exceedance will in fact occur or whether this method has overestimated the exposure because of the uncertainties associated with the screening method.

6.1.4 Potential Groundwater Contributions to Surface Water Chemical Concentrations

Although the potential impact of discharging groundwater on CRLF populations is not explicitly delineated, it should be noted that groundwater could provide a source of pesticide to surface water bodies – especially low-order streams, headwaters, and groundwater-fed pools. This is particularly likely if the chemical is persistent and mobile. Soluble chemicals that are primarily subject to photolytic degradation will be very likely to persist in groundwater and can be transported over long distances. Similarly, many chemicals degrade slowly under anaerobic conditions (common in aquifers) and are thus more persistent in groundwater. Much of this groundwater will eventually be discharged to the surface – often supporting stream flow in the absence of rainfall. Continuously flowing low-order streams, in particular, are sustained by groundwater discharge, which can constitute 100% of stream flow during baseflow (no runoff) conditions. Thus, it is important to keep in mind that pesticides in groundwater may have a major detrimental impact on surface water quality and on CRLF habitats.

SCI-GROW may be used to determine likely 'high-end' groundwater vulnerability, with the assumption (based upon persistence in hypoxic or anoxic conditions, and mobility) that much of the compound entering the groundwater will be transported some distance and eventually discharged into surface water. Although concentrations in a receiving water body resulting from groundwater discharge cannot be explicitly quantified, it should be assumed that significant attenuation and retardation of the chemical will have occurred prior to discharge. Nevertheless, groundwater could still be a significant, consistent source of chronic background concentrations in surface water and may also add to surface runoff during storm events (as a result of enhanced groundwater discharge typically characterized by the 'tailing limb' of a storm hydrograph).

6.1.5 Terrestrial Exposure Modeling of 2,4-D

The Agency relies on the work of Fletcher *et al.* (1994) for setting the assumed pesticide residues in wildlife dietary items. These residue assumptions are believed to reflect a realistic upper-bound residue estimate, although the degree to which this assumption reflects a specific percentile estimate is difficult to quantify. It is important to note that the field measurement efforts used to develop the Fletcher estimates of exposure involve highly varied sampling techniques. It is entirely possible that much of these data reflect residues averaged over entire above-ground plants in the case of grass and forage sampling.

It was assumed that ingestion of food items in the field occurs at rates commensurate with those in the laboratory. Although the screening assessment process adjusts dry-weight estimates of food intake to reflect the increased mass in fresh-weight wildlife food intake estimates, it does not allow for gross energy differences. Direct comparison of a laboratory dietary concentration-based effects threshold to a fresh-weight pesticide residue estimate would result in an underestimation of field exposure by food consumption by a factor of 1.25 – 2.5 for most food items.

Differences in assimilative efficiency between laboratory and wild diets suggest that current screening assessment methods do not account for a potentially important aspect of food requirements. Depending upon species and dietary matrix, bird assimilation of wild diet energy ranges from 23 – 80%, and mammal's assimilation ranges from 41 – 85% (U.S. EPA, 1993). If it is assumed that laboratory chow is formulated to maximize assimilative efficiency (*e.g.*, a value of 85%), a potential for underestimation of exposure may exist by assuming that consumption of food in the wild is comparable with consumption during laboratory testing. In the screening process, exposure may be underestimated because metabolic rates are not related to food consumption.

For the terrestrial exposure analysis of this risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving a treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

6.1.6 Spray Drift Modeling

Although there may be multiple 2,4-D applications at a single site, it is unlikely that the same organism would be exposed to the maximum amount of spray drift from every application made. In order for an organism to receive the maximum concentration of 2,4-D from multiple applications, each application of 2,4-D would have to occur under identical atmospheric conditions (*e.g.*, same wind speed and – for plants – same wind direction), and (if it is an animal) the animal being exposed would have to be present directly downwind at the same distance after each application. Although there may be sites where the dominant wind direction is fairly consistent (at least during the relatively quiescent conditions that are most favorable for aerial spray applications), it is, nevertheless, highly unlikely that plants in any specific area would receive the maximum amount of spray drift repeatedly. It appears that in most areas, based upon available meteorological data, wind direction is temporally variable, even within the same day. Additionally, other factors including variations in topography, cover, and meteorological conditions over the transport distance are not accounted for by the AgDRIFT model (*i.e.*, spray drift from aerial and ground applications in a flat area with little to no ground cover and a steady, constant wind speed and direction is modeled). Therefore, in most cases, the drift estimates from AgDRIFT may overestimate exposure even from single applications, especially as the distance increases from the site of application, since the model does not account for potential obstructions (*e.g.*, large hills, berms, buildings, trees, *etc.*). Furthermore, conservative assumptions are often made regarding the droplet size distributions being modeled (*e.g.*, 'ASAE Very Fine to Fine' for orchard uses and 'ASAE Very Fine' for agricultural uses), the application method (*e.*g., aerial), release heights and wind speeds. Alterations in any of these inputs would change the area of potential effect.

The analysis conducted in this assessment did not include any mitigation resulting from the RED regarding spray drift management requirements. If these conditions were incorporated into the analysis, it it likely that the estimated buffer widths would be reduced.

6.2 Effects Assessment Uncertainties

6.2.1 Age Class and Sensitivity of Effects Thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. The acute toxicity data for fish are collected on juvenile fish between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes *(e.g.,* first instar for daphnids; second instar for amphipods, stoneflies, and mayflies; and third instar for midges).

Testing of juveniles may overestimate toxicity at older age classes for pesticide active ingredients that act directly without metabolic transformation because younger age classes may not have the enzymatic systems associated with detoxifying xenobiotics. In so far as the available toxicity data providing ranges of sensitivity information with respect to age class, this assessment uses the most sensitive life-stage information as measures of effect for surrogate animals and is, therefore, considered as protective of the assessed species.

Additionally, variation in toxicity was observed when temperature or pH was adjusted in a few aquatic studies. For example, one study (E006387) observed an increase in toxicity to *Cyprinus carpio* with corresponding increases in temperature. Another study (E61180) observed increased toxicity with lower pH's; when pH was adjusted to remain between

7.0 and 7.4, the LC_{50} value increased approximately 10-fold. While the studies that produced toxicity values that were used quantitatively generally adhered to guideline test conditions, uncertainties remain of the effects of 2,4-D in the environment given the likelihood for variable temperature, pH, and other conditions.

6.2.2 Use of Surrogate Species Effects Data

Freshwater fish are used as surrogate species for aquatic-phase amphibians. Although, acute amphibian data are available for 2,4-D (ester and acid), the available open literature information on 2,4-D toxicity to aquatic-phase amphibians shows that acute ecotoxicity endpoints for aquatic-phase amphibians are generally about 7 times less sensitive than freshwater fish for exposure to acid /salts (common carp LC_{50} = 24.15 mg a.e./L; Western chorus frog tadpoles LC_{50} = 181 mg a.e./L) and 2 times less sensitive than freshwater fish for exposure to esters (bluegill sunfish LC_{50} = 0.26 mg a.e./L; leopard frog tadpoles LC_{50} = 0.505 mg a.e./L). Therefore, endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CRLF, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CRLF is likely to overestimate the potential risks to those species. Efforts are made to select the organisms most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

As previously discussed in the aquatic toxicity portion of this assessment (**Section 4.2**), aquatic amphibian data were submitted by the registrant and available in the open literature. However, since these data were less sensitive than the freshwater fish data and it is unknown where the CRLF falls on a sensitivity distribution for amphibians or for aquatic vertebrate species, EFED selected the most sensitive aquatic vertebrate toxicity test for risk estimation. For further characterization, EFED also calculated acute RQs using the most sensitive amphibian data for acid/salts and for esters (discussed in **Section 5.2.1.1**).

Acceptable guideline toxicity tests and open literature studies for reptiles are not currently available for quantitative use to assess potential risks of 2,4-D use in California to the AW. Therefore, toxicity data for surrogate species (*i.e.*, birds for reptiles) are used in some instances to assess risks. Efforts are made to select the organisms, which are most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

6.2.3 Sublethal Effects

When assessing acute risk, the screening-level risk assessment relies on the acute mortality endpoint as well as a suite of sublethal responses to the pesticide, as determined by the testing of species response to chronic exposure conditions and subsequent chronic risk assessment. Consideration of additional sublethal data in the effects determination is exercised on a case-by-case basis and only after careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishing a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints. However, the full suite of sublethal effects from valid open literature studies is considered for the purposes of defining the action area.

Although the full suite of sublethal endpoints potentially available in the effects literature (regardless of their significance to the assessment endpoints) are often considered to define the action area for other chemicals, in the case of 2,4-D, LOC exceedances are expected to occur on all land cover types throughout the state of California as a result of this federal action and the final full extent of the action area is assumed to encompass the entire state. To the extent to which sublethal effects are not considered in this assessment, the potential direct and indirect effects of 2,4-D on listed species may be underestimated.

A detailed spreadsheet of the available ECOTOX open literature data, which includes the full suite of sublethal endpoints, is presented in **Appendix G**.

7. Risk Conclusions

In fulfilling its obligations under Section 7(a)(2) of the Endangered Species Act, the information presented in this endangered species risk assessment represents the best data currently available to assess the potential risks of 2,4-D to the CRLF and AW and their designated critical habitats.

Based on the best available information, the Agency makes a "may affect and likely to adversely affect" (LAA) determination for both the CRLF and the AW for all assessed use of 2,4-D except Citrus and Potatoes; the LAA is based on both direct and indirect effects to the CRLF and AW. For Citrus and Potatoes, the Agency makes a "may affect but not likely to adversely affect" (NLAA) determination for both the CRLF and AW from the assessed uses of 2,4-D:

- Although the mammalian dose-based chronic LOC was exceeded for both the CRLF and the AW prey, EFED determined that this effect would be insignificant as the potential small effect on mammal reproduction (as prey of the CRLF and AW) would not likely impact the overall prey base. It is anticipated that any effects would be small since the RQs only mildly exceeded the LOC.

- Although the avian acute dose-based LOC was exceeded for AW prey, EFED determined that this effect was discountable and insignificant as the predicted percentage of acute effect was only 0.0033% of the bird population (birds as prey items of the AW), and if even if this effect did occur, the overall prey base of the AW would likely not be affected.
- Although the terrestrial plant LOC was exceeded for both CRLF and the AW, EFED determined the effect to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.

Based on potential for effects across several taxa, all currently registered uses of 2,4-D in California except Citrus and Potatoes have the potential to cause indirect effects to the CRLF and the AW. Additionally, the Agency has determined that there is potential for modification of the designated critical habitat of the CRLF for all assessed uses of 2,4-D except Citrus and Potatoes based on effects to terrestrial and aquatic plants. For the AW, based on effects to terrestrial plants, all relevant (risks of aquatic weed control uses to terrestrial plants were not estimated) assessed uses of 2,4-D except Citrus and Potatoes have the potential to modify the designated critical habitat. Both species may experience modification of their designated critical habitats through reduction of prey items. Given the LAA determinations for the CRLF and the AW for all but two assessed uses and potential modification of designated critical habitat for all but two uses, a description of the baseline status and cumulative effects for the CRLF is provided in **Attachment 2** and the baseline status and cumulative effects for the AW is provided in **Attachment 4**.

A summary of the risk conclusions and effects determinations for the CRLF and AW and their critical habitats, given the uncertainties discussed in **Section 6**, is presented in **Tables 7.1** and **7.2**.

Table 7.1 Effects Determination Summary for the Effects of 2,4-D on the CRLF and AW		
Assessment Endpoint	**Effects Determination** [1]	**Basis for Determination**
Survival, growth, and/or reproduction of CRLF individuals	LAA[2]	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): Freshwater fish data used as surrogate for CRLF. *Adult survival*: Acute LOC was exceeded in the aerial forestry, tree and brush control drift+runoff ester uses and all direct application to water scenarios. The chance of individual effects (*i.e.*, mortality) for freshwater fish (surrogate for aquatic-phase CRLFs) is as high as ~1 in 1 for direct water applications. Out of 26 incidents reported for aquatic organisms for 2,4-D acid and DMA salt, 7 registered uses were reported with certainties of highly probable(2), probable(2) and possible (2). Incidents for 2,4-D were filed on aquatic organisms from runoff or drift. Use sites for the above incidents were reported on home/lawn, corn, agricultural areas, rights of way/railroad, lake, pond, stream, turf/golf course. *Growth and reproduction:* Chronic LOC was not exceeded for any scenarios.
		Terrestrial-phase (Juveniles and Adults): Avian data used as surrogate for CRLF. *Survival:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for granular applications. The chance of individual effects (*i.e.*, mortality) for terrestrial-phase CRLF (Avian data used as surrogate for CRLF) is as high as ~1 in 1 for direct water applications (ditchbanks), non-cropland, forestry, tree and brush control, and grass grown for sod applications. Based on one incident report from runoff, 2,4-D has been implicated as being toxic to birds with probable certainty for a use of undetermined legality. *Growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbanks) for liquid applications derived from T-REX and T-HERPS modeled scenarios.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity *Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios. *Vascular aquatic plants*: LOC was exceeded for several acid/salt use scenarios and all direct application to water scenarios. *Freshwater invertebrates*: Acute LOC was exceeded for all direct application to water scenarios. Based on the results of probit analysis, there is a significant chance (> 10%) that direct applications to water (aquatic weed control ester uses) will impact prey of the CRLF via direct effects on aquatic invertebrates as dietary food items. *Freshwater fish*: Acute LOC was exceeded for aerial forestry, tree and brush control, and all direct application to water scenarios. Based on the results of

		probit analysis, there is a significant chance (> 10%) that direct applications to water will impact prey of the CRLF via direct effects on freshwater fish as dietary food items. Out of 26 incidents reported for aquatic organisms for 2,4-D acid and DMA salt, 6 registered uses were reported with certainties of highly probable(2), probable(2) and possible (2). Incidences for 2,4-D were filed on aquatic organisms from runoff or drift. Use sites for the above incidents were reported on home/lawn, corn, agricultural areas, rights of way/railroad, lake, pond, stream, turf/golf course.
		Terrestrial prey items, riparian habitat *Terrestrial invertebrates*: Acute LOC for small insects was exceeded for all scenarios except citrus and potatoes. Acute LOC for large insects was exceeded for several scenarios. *Terrestrial-phase amphibians, acute toxicity*: Acute LOCs were exceeded in all T-REX and T-HERPS modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for T-REX modeled granular applications. The chance of individual effects (*i.e.*, mortality) for terrestrial-phase CRLF (Avian data used as surrogate for CRLF) is as high as ~1 in 1 for direct water applications (ditchbanks), non-cropland, forestry, tree and brush control, and grass grown for sod applications. *Terrestrial-phase amphibians, growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbank exposure) for liquid application. *Small terrestrial mammals, acute toxicity:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod and all direct water application scenarios (ditchbanks) for granular applications. Based on the results of probit analysis, there is a significant chance (> 10%) that several of the 2,4-D uses will impact prey of the CRLF via direct effects on mammals as dietary food items. Based on three incident reports, 2,4-D has been implicated as being toxic to mammals with possible and probable certainty for registered and undetermined use legalities. *Small terrestrial mammals, growth and reproduction:* For liquid applications of 2,4-D, chronic dose-based LOCs were exceeded for all application scenarios. Chronic-dietary based RQ values exceeded the LOC for all liquid application scenarios except potatoes and citrus. *Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. 140 of these incidents were registered uses and 143 were of unknown legality. The majority

		of the reports were of possible to highly probable certainty. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover.
Survival, growth, and/or reproduction of AW individuals	LAA[2]	**Potential for Direct Effects**
		Terrestrial-phase (Juveniles and Adults): Avian data used as surrogate for AW. *Survival:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for granular applications. The chance of individual effects (*i.e.*, mortality) for AW (Avian data used as surrogate for AW) is as high as ~1 in 1 for direct water application (ditchbanks). Based on one incident report 2,4-D, has been implicated as being toxic to birds with probable certainty for an undetermined use legality. *Growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbanks) for liquid application.
		Potential for Indirect Effects
		Terrestrial prey items, riparian habitat *Terrestrial invertebrates*: Acute LOC for small insects was exceeded for all scenarios except citrus and potatoes. Acute LOC for large insects was exceeded for several scenarios. *Terrestrial-phase amphibians, acute toxicity*: Acute LOCs were exceeded in all T-REX and T-HERPS modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for T-REX modeled granular applications. The chance of individual effects (*i.e.*, mortality) for terrestrial-phase CRLF (Avian data used as surrogate for CRLF) is as high as ~1 in 1 for direct water application (ditchbanks). *Terrestrial-phase amphibians, growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbank exposure) for liquid application. *Small terrestrial mammals, acute toxicity:* Acute LOC was exceeded in all modeled scenarios except citrus and potatoes for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbanks) for granular applications. Based on the results of probit analysis, there is a significant chance (> 10%) that several of the 2,4-D uses will impact prey of the AW via direct effects on mammals as dietary food items. Based on three incident reports, 2,4-D has been implicated as being toxic to animals with possible and probable certainty for registered and undetermined use legalities. *Small terrestrial mammal, growth and reproduction*: For liquid applications of 2,4-D, chronic dose-based LOCs were exceeded for all application scenarios.

		Chronic-dietary-based RQ values exceeded the LOC for all liquid application scenarios except potatoes and citrus. *Birds, acute toxicity:* Acute LOC was exceeded in all modeled scenarios for liquid applications. Acute LOC was exceeded in field corn, popcorn, sweet corn, grain or forage sorghum, non-cropland, ornamental turf, grass grown for sod, and all direct water application scenarios (ditchbank exposure) for granular applications. Based on the results of probit analysis, there is a significant chance (> 10%) that all uses except potatoes and citrus uses will impact prey of the AW via direct effects on birds as dietary food items. Based on one incident report, 2,4-D has been implicated as being toxic to animals with probable certainty for an undetermined use legality. *Birds, growth and reproduction*: Dietary-based chronic RQ values exceeded the LOC at 1 app @ 54 lb a.e./acre for aquatic weed control (ditchbank exposure) for liquid application. *Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. 140 of these incidents were registered uses and 143 were of unknown legality. The majority of the reports were of possible to highly probable certainty. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover.

[1]No effect (NE); May affect but not likely to adversely affect (NLAA); May affect and likely to adversely affect (LAA)

[2] The LAA call is for all usese except Citrus and Potatoes. For both Citrus and Potatoes for both species (CRLF and AW), a NLAA call was made by EFED. For Citrus and Potato, the LOC was exceeded for several indirect effects: (1) mammals as prey (chronic, CRLF and AW), (2) birds as prey (acute, AW only), and (3) terrestrial plants (CRLF and AW). The reasons for the NLAA calls are listed below:

- Although the mammalian dose-based chronic LOC was exceeded for both the CRLF and the AW prey, EFED determined that this effect would be insignificant as the potential small effect on mammal reproduction (as prey of the CRLF and AW) would not likely impact the overall prey base. It is anticipated that any effects would be small since the RQs only mildly exceeded the LOC.
- Although the avian acute dose-based LOC was exceeded for AW prey, EFED determined that this effect was discountable and insignificant as the predicted percentage of acute effect was only 0.0033% of the bird population (birds as prey items of the AW), and if even if this effect did occur, the overall prey base of the AW would likely not be affected.
- Although the terrestrial plant LOC was exceeded for both CRLF and the AW, EFED determined the effect to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.

Table 7.2 Effects Determination Summary for Critical Habitat Impact Analysis			
Species	**Assessment Endpoint**	**Effects Determination** [1]	**Basis for Determination**
CRLF	Modification of aquatic-phase PCE	HM[2]	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. 140 of these incidences are registered uses. *Non-vascular aquatic plants*: LOC was exceeded for all direct surface aquatic weed control scenarios. *Vascular aquatic plants*: LOC was exceeded for several acid/salt use scenarios and all direct application to water scenarios. There is a potential for direct effects to aquatic-phase CRLF and indirect effects via reduction of aquatic-phase prey items (aquatic invertebrates, fish, and aquatic-phase amphibians) as described in **Section 5**.
	Modification of terrestrial-phase PCE	HM[2]	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. 140 of these incidences are registered uses. There is a potential for direct effects to terrestrial–phase CRLF and indirect effects via reduction of terrestrial-phased prey items (mammals, terrestrial invertebrates, and frogs) as described in **Section 5**.
AW	Modification of terrestrial-phase PCE	HM[2]	*Terrestrial plants*: LOCs were exceeded for monocots for all modeled scenarios except citrus and potatoes. LOCs were exceeded for dicots for all modeled scenarios. For 2,4-D, 358 incidents were reported for mostly plant damage to a wide variety of terrestrial plants particularly from direct treatment or spray drift. Other reported incident exposures included spills, stunted growth, discoloration, runoff, persistence in crop and carryover. 140 of these incidences are registered uses. There is a potential for direct and indirect effects to the AW via reduction of terrestrial-phased prey items (mammals, birds, terrestrial invertebrates, and frogs) as described in **Section 5**.

[1]Habitat modification (HM) or No effect (NE)
[2] The HM call is for all usese except Citrus and Potatoes. For both Citrus and Potatoes for both species (CRLF and AW), a NE call was made by EFED. For Citrus and Potato, the LOC was exceeded for several indirect effects: (1) mammals as prey (chronic, CRLF and AW), (2) birds as prey (acute, AW only), and (3) terrestrial plants (CRLF and AW). The reasons for the NE calls are listed below:

- Although the mammalian dose-based chronic LOC was exceeded for both the CRLF and the AW prey, EFED determined that this effect would be insignificant as the potential small effect on mammal reproduction (as prey of the CRLF and AW) would not likely impact the overall prey base. It is anticipated that any effects would be small since the RQs only mildly exceeded the LOC.

- Although the avian acute dose-based LOC was exceeded for AW prey, EFED determined that this effect was discountable and insignificant as the predicted percentage of acute effect was only 0.0033% of the bird population (birds as prey items of the AW), and if even if this effect did occur, the overall prey base of the AW would likely not be affected.
- Although the terrestrial plant LOC was exceeded for both CRLF and the AW, EFED determined the effect to be insignificant as the potential small effect on the vegetation would likely not impact the overall habitat quality. It is anticipated that any effects would be small as the RQs only mildly exceeded the LOC.

Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated.

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (*i.e.*, food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (*i.e.*, attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF and AW life stages within the action area and/or applicable designated critical habitat. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the assessed species.
- Quantitative information on prey base requirements for the assessed species. While existing information provides a preliminary picture of the types of food sources utilized by the assessed species, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment

immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual species and potential modification to critical habitat.

8. References

Anderson, A.M, Byrtus, G., Thompson J., Humphries, D., Hill, B., and Bilyk, M., 2002. Baseline Pesticide Data for Semi-Permanent Wetlands in the Aspen Parkland of Alberta. Albeta Environment, Publication No. T/673.

Feitshans, T.A., 1999. An Analysis of State Pesticide Drift Laws, San Joaquin Agric. L. Rev. 1, 37 (Spring 1999).

Fletcher, J.S., J.E. Nellessen, and T.G. Pfleeger. 1994. Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, and instrument for estimating pesticide residues on plants. Environmental Toxicology and Chemistry 13 (9):1383-1391.

Gibson, L. R. and M. Liebman. 2002. Course Material for *Principles of Weed Science*, Agronomy 317, Iowa State University. Website accessed 15 July 2003, http://www.agron.iastate.edu/courses/Agron317/Herbicide_mode_of_action.htm.

Harrison, S.A., Watschke, T.L., Mumma, R.O., Jarrett, A.R., and Hamilton, G.W. Jr. 1993. Nutrient and pesticide concentrations in water from chemically treated turfgrass. in K. Racke and A. Leslie (editors), Pesticides in urban environments: Fate and significance. American Chemical Society (ACS) Symposium Series 1993, #522, p. 191-207.

Hayes and Tennant. 1985. Diet and feeding behavior of the California red-legged frog. The Southwestern Naturalist 30(4): 601-605.

Hoerger, F., and E.E. Kenaga. 1972. Pesticide residues on plants: Correlation of representative data as a basis for estimation of their magnitude in the environment. In F. Coulston and F. Korte, *eds*., Environmental Quality and Safety: Chemistry, Toxicology, and Technology, Georg Thieme Publ, Stuttgart, West Germany, pp. 9-28.

Majewski , M.S. and P.D. Capel. 1995. Pesticides in the atmosphere: distribution, trends, and governing factors. Ann Arbor Press, Inc. Chelsea, MI.

Neher, L.A., T.M. Mischke, and R.J. Oshima. 1980. Monitoring for the Presence of 2,4-D in the Air: A Cooperative California Study

Paris, D.F., Steen, W.C., Baughman, G.L., and Barrnett, J.T. Jr, 1981. Second-Order Model to Predict Microbial Degradation of Organic Compounds in Natural Waters. Applied and Environmental Microbiology, vol. 41, No. 3, p 603-609.

Rawn, D.F.K., Halldorson, T. H. J.., Lawson, B.D. and Muir, C.G. 1999. A Multi-Year Study of Four Herbicides in Air and Precipitation from a Small Prairie Watershed. J. Environ. Qual. 28:898-906.

Simpson, S.W., L.A. Neher, T.M. Mischke, and R.J. Oshima. 1980. Monitoring the Air for the Presence of 2,4-D in Kern County, Kings County and San Luis Obispo County. A Cooperative California Study

Smith, A.E. and Hayden, B.J. 1980. Hydrolysis of MCPA Esters and the Persistence of MCPA in Saskatchewan Soils. Bull. Environm. Contam. Toxicol., 25, 369-373.

Swarzenbach, R.P., Gschwend, P.M., and Imboden, D.M., 1993. Environmental Organic Chemistry. John Wiley and Sons, Inc. New York.

U.S. Environmental Protection Agency (USEPA). 1993. Wildlife Exposure Factors Handbook. Volume I of II. EPA/600/R-93/187a. Office of Research and Development, Washington, D. C. 20460. EPA/600/R-93/187a.

U.S. EPA (Environmental Protection Agency) (2006) An inventory of sources and environmental releases of dioxin-like compounds in the United States for the years 1987, 1995 and 2000. National Center for environmental assessment, Washington DC; EPA/600P-03/002F. Available from: National Technical Information Service, Springfield, VA, and online at http://epa.gov/ncea.

U.S. EPA. 2005 Reregistration Eligibility Decision for 2,4-D, Prevention , Pesticides and toxic substances (7508C) EPA 738-R-05-002 June 2005

U.S. Environmental Protection Agency (U.S. EPA). 1998. Guidance for Ecological Risk Assessment. Risk Assessment Forum. EPA/630/R-95/002F, April 1998.

U.S. EPA. 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs. Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004.

USFWS. 2002. Recovery Plan for the California Red-legged Frog (*Rana aurora draytonii*). Region 1, USFWS, Portland, Oregon. (http://ecos.fws.gov/doc/recovery_plans/2002/020528.pdf)

USFWS. 2006. Endangered and threatened wildlife and plants: determination of critical habitat for the California red-legged frog. 71 FR 19244-19346.

USFWS. Website accessed: 30 December 2006. http://www.fws.gov/endangered/features/rl_frog/rlfrog.html#where

U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS). 1998. Endangered Species Consultation Handbook: Procedures for Conducting Consultation and Conference Activities Under Section 7 of the Endangered Species Act. Final Draft. March 1998.

USFWS/NMFS. 2004. 50 CFR Part 402. Joint Counterpart Endangered Species Act Section 7 Consultation Regulations; Final Rule. FR 47732-47762.

U.S. Fish and Wildlife Service (USFWS). 1997. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for the Callippe Silverspot Butterfly and the Behren's Silverspot Butterfly and Threatened Status for the Alameda Whipsnake. Available online at: http://ecos.fws.gov/docs/federal_register/fr3183.pdf (Accessed on April 17, 2008).

U.S. Fish and Wildlife Service (USFWS). 2003. Draft Recovery Plan for Chaparral and Scrub Community Species East of San Francisco Bay, California. U.S. Fish and Wildlife Service, Region 1, Portland, Oregon. 306 pages. Available online at: http://ecos.fws.gov/docs/recovery_plans/2003/030407.pdf (Accessed on April 17, 2008

USFWS. 2002. Recovery Plan for the California Red-legged Frog (*Rana aurora draytonii*). Region 1, USFWS, Portland, Oregon. (http://ecos.fws.gov/doc/recovery_plans/2002/020528.pdf)

U.S. Fish and Wildlife Service (USFWS). 2005. Species Account: Alameda Whipsnake (*Masticophis lateralis euryxanthus*). Sacramento Fish and Wildlife Office. Available online at: http://www.fws.gov/sacramento/es/animal_spp_acct/alameda_whipsnake.pdf (Accessed on April 17, 2008).

U.S. Fish & Wildlife Service. 2006. Endangered and Threatened Wildlife and Plants; Designation of Critical Habitat for the Alameda Whipsnake; Final Rule. Available online at: http://edocket.access.gpo.gov/2006/pdf/06-8367.pdf (Accessed on April 17, 2008).

Westphal, M. 1998. Alameda Whipsnake: The Fastest Snake in the West. Tideline. Vol. 18 No. 2 1-3. Available online at: http://www.fws.gov/desfbay/Archives/Whip/Whip.htm (Accessed on October 3, 2007).

Willis, G.H. and L.L. McDowell. 1987. Pesticide Persistence on Foilage in Reviews of Environmental Contamination and Toxicology. 100:23-73.

Wolfe, N.L., M.E-S. Metwally and A.E. Moftah. 1989. Hydrolytic Transformations of Organic Chemicals in the Environment. In: Reactions and Movement of Organic Chemicals in Soils. B.L. Sawhney and K. Brown, (Eds), Soil Science Society of America and American Society of Agronomy, Madison, WI. p. 229-242.

Wolfe, N.L. 1990. Abiotic Transformations of Toxic Organic Chemicals in the Liquid Phase and Sediments. In: Toxic Organic Chemicals in Porous Media. Z. Gerstl, Y. Chen, U. Mingelgrin and B. Yaron. (Eds.). Springer-Verlag, New York. p. 136-147.